Table of Contents

ASHI Quarterly

American Society for Histocompatibility and Immunogenetics

Volume 26, Number 2

Features

SCIENTIFIC COMMUNICATIONS

HLA and Platelet Transfusion Outcome 56
HLA Matchmaker: A Molecularly Based Algorithm for Histocompatibility Determination 60
Polymorphism and Antigenicity of HLA-MICA 64
Diversity of NK Cell Receptors and their HLA class I Ligands 68

HIGHLIGHTS

Tech Tips: Effect of Thymoglobulin on Immune Monitoring and Crossmatch Interpretation 74
Summary of the Northeast Regional Education Workshop 75
2002 ASHI Officers and Council Members 76
New Members 78

Departments

EDITORIAL

Letter from the Editor-in-Chief 46
President's Column 50
A National Conference to Analyze the Wait List for Kidney Transplantation 53

COMMITTEE ACTIVITIES

ASHI Web Highlights 84
Accreditation News 86

IN MEMORIAM

J. Marilyn MacQueen 73

ASHI QUARTERLY CONTINUING EDUCATION QUIZ 66

Copy Editor's Note

Individuals interested in submitting articles for the ASHI Quarterly should observe the following requirements:

- All articles must be submitted on a floppy disk or via e-mail in Microsoft Word format
- Include a hard copy of the article, typed and double-spaced

Article submissions should be forwarded to:

ASHI Quarterly
Jennifer Szwalek
17000 Commerce Parkway, Suite C
Mount Laurel, NJ 08054
E-mail: jszwalek@ahint.com

Copy deadlines for future issues

Third Quarter 2002:
August 1, 2002

Fourth Quarter 2002:
November 8, 2002

Editor-in-Chief
Judith E. Baker, CHS, I(ASCP)

Assistant Editor
Jennifer Szwalek

Section Editors
Francesco M. Marincola, MD
Basic Science

Steven K. Takemoto, PhD
Clinical Science

Patty Saiz, CHS, CHT
Technological Issues

Executive Director
Len Morrissey

Copy Editor
Sohini Mitra

Graphic Designer
Mark Denato

ASHI Quarterly is published quarterly by the American Society of Histocompatibility and Immunogenetics, 17000 Commerce Parkway, Mount Laurel, New Jersey 08054. Subscription rates are $35 within the United States and Canada, $40 outside the U.S. and Canada. Subscriptions are accepted on a calendar year basis. Remittance should be made by check, draft or money order in U.S. currency, payable to the American Society for Histocompatibility and Immunogenetics. Notify ASHI of change of address at least 30 days before date of issue by sending both the old and new address. Multiple reproductions of any material in this publication is permitted only under license from the American Society of Histocompatibility and Immunogenetics with the written permission of the authors. Fair use of such material by individual readers and non-profit libraries, such as making single copies of an article for use in teaching or research, is allowed. Consent of the authors and ASHI must be obtained to reprint figures, tables or excerpts from the printed matter. The statements and opinions are not necessarily those of ASHI. ASHI does not endorse any products.

Copyright © 2002. American Society of Histocompatibility and Immunogenetics.

www.ashi-hla.org

Letter From the Editor-in-Chief

Judith E. Baker, CHS, I(ASCP)

The editorial staff of the ASHI Quarterly is pleased to present to you, the readers, the Second Quarter issue. We hope that you will find the articles in this issue interesting, thought-provoking and, in some cases, useful in your day-to-day practice of histocompatibility and immunogenetics.

This time of the year seems to be particularly busy with many deadlines looming in the immediate future and many of the members preparing for the ASHI Regional workshops and the IHWC. I would like to thank everyone who contributed to this issue of the Quarterly. It represents a significant investment of time on behalf of each of the authors but it also benefits those of us that have the opportunity to read, enjoy and learn from each of the articles.

Hopefully there is something for everyone in this issue. You will learn about "HLA and Platelet Transfusion Outcome" and some interesting research that is continuing in this area. You will be given a personal tour of the "HLA Matchmaker" that is available on the ASHI web site for all of us to use. The article on "Polymorphism and Antigenicity of HLA-MICA" is a wonderful overview of this system and goes well with the article on the "Diversity of NK Cell Receptors and Their HLA Class I Ligands." Perhaps the use of thymoglobulin has interfered with your ability to perform immune monitoring or crossmatching post transplant. If so, be sure and read the "Tech Tips" section in this issue. For those of you interested in the wait list for kidney transplantation, the editorial by Steve Takemoto, the Clinical Science Editor, will give you a summary of a meeting held in March 2002 to discuss the ever-expanding list of patients awaiting renal transplantation. Dr. Bray also discusses this meeting in the President's Column. As always the Web Highlights provide a continuation of the "tour" of the web site looking at the "About ASHI", "Accreditation" and "ASHI Governance" areas. Dr. Land has included an overview of the April ARB meeting along with other pertinent information regarding accreditation. Lori Osowski and John Hart were kind enough to provide a summary of the Northeast Regional Workshop held in Baltimore, MD. It was short notice to write the summary and they did a great job.

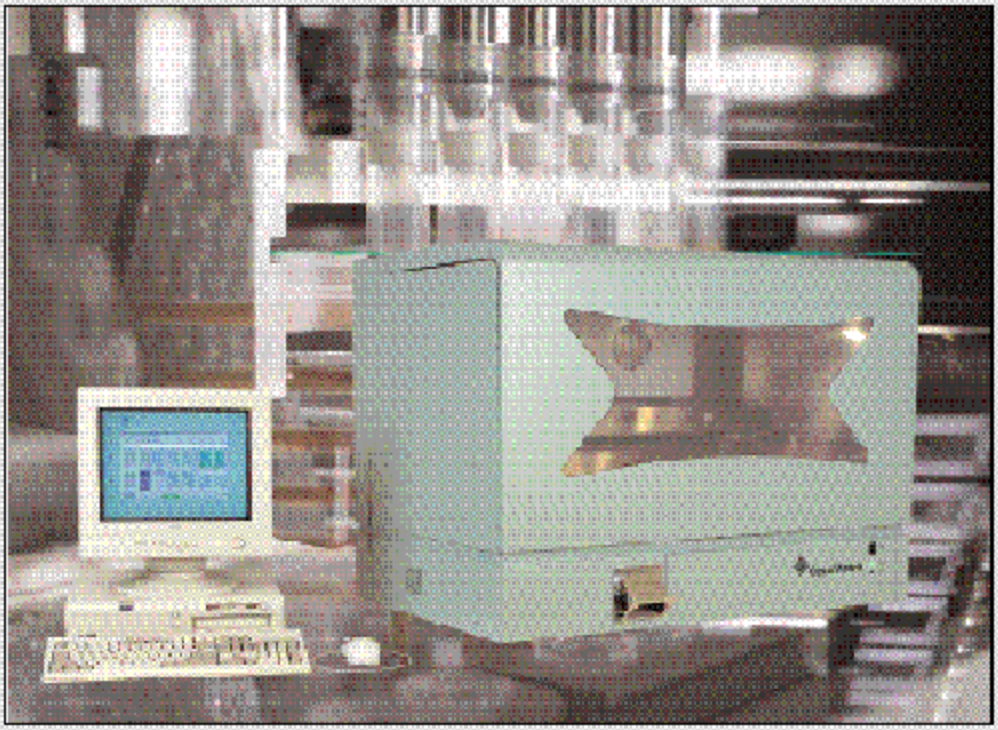
Once again I would like to invite input from each of you as you take time to read the articles in this issue. We welcome your comments. We would like to know what you like, what you don't like, what we can change, what articles you would like to see in future issues, etc. Send us your questions and we'll try to get them answered in the next issue. Send us your articles and we'll publish them. Send us your suggestions for articles and we'll try to find experts to write the articles. Get involved!! This is your publication so let's make it a publication "for the membership, by the membership."

For those of you wanting Continuing Education credits, don't forget to take the quiz and share it with others who may not receive copies of the ASHI Quarterly. This issue also contains information for the ASHI 28th Annual Meeting to be held in Nashville in October. Keep your eye out for registration material, which you will receive shortly. Mark your calendars for October 19-23, 2002.

GenoM™-48

Introducing Automated Nucleic Acid Isolation and Purification

Easy, Reliable and Flexible for HLA Typing



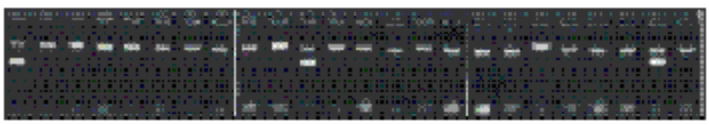
True Walk-Away Automation

WIDE RANGE OF APPLICATIONS:

- Genomic DNA
- Total RNA
- mRNA
- Plasmid DNA
- Viral RNA
- PCR Clean-Up

GenoM™-48 Fully Automated Nucleic Acid Isolation and Purification

DNA, isolated and purified using the GenoPrep™ DNA from Blood Kit and the GenoM™-48, was successfully amplified using the OLERUP SSP™ DR-Low Resolution Kit.



Gel electrophoresis of DNA amplified using OLERUP SSP™ DR-Low Resolution Kit. The result is DRB1*01xx, 0901 DRB4.

GenoVision Inc.
[illegible] South Bolmar St., Suite R
West Chester, PA [illegible] USA
[illegible]
Ordres: +1 [illegible]
Fax: +1 [illegible]

GenoVision AB
Fryejordan 40
N-[illegible] Oslo, Norway
[illegible]
Ordres: +47 [illegible]
Fax: +47 [illegible]

GenoVision Vertriebsgesellschaft
Löwengasse 47/[illegible]
A-[illegible] Vienna, Austria
office@genovision.at
Ordres: +43 1 710 [illegible]
Fax: +43 1 710 [illegible]

GenoVision

www.genovision.com

QIAamp® Next Generation

The new standard for diagnostic PCR sample preparation

Today, many leading clinical laboratories and diagnostic companies use and recommend QIAamp® technology as the standard nucleic acid purification system for their molecular assays.

Quality and reliability

- Reproducible and consistent results — fewer failures for improved productivity
- Enhanced performance — in PCR, RT-PCR, NASBA®, LCR, SDA, TMA, or genotyping

Universal system for all your routine assays

- Many options — DNA and RNA from a wide range of samples
- Highly compatible — one prep for multiple assays and multiple targets

Set the standard in your lab — choose QIAamp Kits for your sample preparation

For more information, visit our web site at www.qiagen.com/clinical/or call QIAGEN today!

QIAamp Kits are intended as general-purpose devices that may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries. The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. Trademarks: QIAGEN®, QIAamp®(QIAGEN); NASBA®(Organon Teknika).

www.qiagen.com

QIAGEN:

Australia
Tel. 03-9840-9800
Fax. 03-9840-9888

Canada
Tel. 800-572-9613
Fax. 800-713-5961

France
Tel. 01-60-920-930
Fax. 01-60-920-925

Germany
Tel. 02103-892-111
Fax. 02103-892-333

Italy
Tel. 02-334-30-11
Fax. 02-334-30-222

Japan
Tel. 03-5547-0811
Fax. 03-5547-0818

Switzerland
Tel. 061-319-30-31
Fax. 061-319-30-33

UK and Ireland
Tel. 01293-422-911
Fax. 01293-422-922

USA
Tel. 800-426-8157
Fax. 800-718-2056

Distributors:

Argentina Biotools S.A. (54 1) 4555-5555 Australia Ritverc VWR International (Kimble) (03) 9330 3500 Belgium Bioconsulting Westburg bv 0800-1-0615 Brazil Laborclin ou Biotec (041) 338-8444 Chile Olen Ltda. Company Limited (02) 2-777-2570 Denmark VWR International A/S (43) 95 07 00 Egypt Cairo 22 57 21 E. Finland VWR International Oy (09) 804 551 Greece Biotechnica S.A. (01) 6749 111 Hellas Kimble (01) 6749 111 or (01) 7612 [illegible] Hong Kong Diagnostic Tech. Co. (02) 8909-4115 or 1-800 90 34 30 Hungary Kft. Labogroup, Inc. (06) 1-343-87 87 Malaysia Biotech Biotechnology Sdn. Bhd. (03) 7954 3161 or (03) 7954 3162 Mexico Química Clínica Marcer S.A. de C.V. (55) 45-67-29 The Netherlands Westburg bv. 0318-550-550 New Zealand Biolab Scientific Ltd. (09) 980 6700 or 0800-655-22 Norway VWR International AS (67) 54 05 00 Poland Diagnostyka Polska Sp.z.o.o. (071) 351 41 60 or (071) 72 70 57 Portugal Izasa Portugal, Lda. (01) 355 38 00 Russia Diagnostic Systems Ltd. (095) 234-4184 Singapore Biotech Pacific (65) 7747 866 Slovakia Labogroup Slovakia spol. s.r.o. 0800-0266 Slovenia Biotehna Biotehna Trade Marketing Pvt. Ltd. (011) 671 5788 South Africa Kimble, S.A. (pty) (011) 975-1830 Sweden VWR International AB (08) 621 34 00 Taiwan Bioman Corporation (02) 2721 2118 Thailand Bacto Trading Co. Ltd. (02) 412-5672 Turkey Medikim Ltd. Sti. (0212) 212 57 50 United Arab Emirates Gulf Medical Projects Corp. (04) 351 9091 USA Research Diagnostics Inc. (973) 539-8300 or (800) 631-5557 Venezuela Química Clínica Marcer C.A. (02) 285-1667 Vietnam Thai Nguyen Import Export Company (028) 822 3908 Zimbabwe Varichem (Pvt) Ltd. (04) 486 311 Other countries: QIAGEN GmbH, Germany

2572 Lymphoprep 7x10 4/30/02 9:33 AM Page 1

FOR PURE LYMPHOCYTE SUSPENSIONS

WHEN YOU THINK "FICOLL-HYPAQUE"... BE ACCURATE!

LYMPHOPREP®

pyrogen-tested

from Nycomed.

LYMPHOPREP® is the ONLY ready-made Metrizoate based Solution for Lymphocyte Isolation according to Bøyum's original work.

Ficoll® is a registered trademark of Pharmacia, Inc.

300 SHAMES DRIVE, WESTBURY, NY 11590 USA • FAX: 1.516.997.4948
WESTBURY, NY: 516.333.2221 1.800.645.6264 (New York Office)
SAN DIEGO, CA: 619.296.9945 1.800.255.9378 (West Coast Office)
info@accuratechemical.com www.accuratechemical.com

ACCURATE CHEMICAL & SCIENTIFIC CORPORATION

IT'S MORE THAN A NAME - IT'S A PROMISE

©2002 Accurate Chemical & Scientific Corp.

EDITORIAL

President's Column

Robert A. Bray, PhD, D(ABHI)

Greetings to all ASHI members, friends and colleagues.

ASHI has been well underway this year with many interesting things in the works and on the horizon. We have completed the transition to the new management company and are slowly moving forward. As with any new endeavor, there have been a few "bumps in the road." However, we have been working very hard to iron out these wrinkles and move forward.

One of the more interesting events that have taken place since my last letter was the much talked-about "National Conference to Analyze the Waitlist." This conference was co-sponsored by AST, ASTS and CMS with ASHI as an invited participant. The conference took place on March 3-5 in Philadelphia, PA.

The goal of the conference was to review the practices surrounding the renal transplant waitlist. The conference was divided into four working groups that focused on the following issues: 1) Access to the list. 2) Assigning Priority on the Waitlist. 3) Management of the Waitlist. 4) Expanded Criteria Donor Kidneys. Several ASHI members were in attendance and they included: Dr. Dolly Tyan, Dr. Steve Takemoto, Dr. Ron Kerman, Dr. Dan Cook, Dr. Mike Cecka as well as myself. ASHI members were placed on two of the groups: Management of the Waitlist (Drs. Kerman and Cecka) and Assigning Priority on the Waitlist (Drs. Tyan, Cook, Takemoto and myself). Both working groups provided for interesting discussions.

Not unexpectedly, one of the main issues that arose in working group #2 was the role/contribution of HLA points to organ allocation. The two issues that were discussed were equity and utility. The definition of equitable is "fair and impartial" while the definition of utility is "useful and beneficial." Thus, was the challenge of our group, to discuss a balance between equity and utility.

We first began by discussing the extremes; Egalitarianism and Utilitarianism. Egalitarianism is the term used to describe a system that uses equity in access to an organ as the only measure of success. The best example of such a system would be a lottery. In this system, each individual has an equal chance at obtaining an organ independent of any bias. By contrast, a total utilitarian view would use outcomes as the only measure for success. In this system, there would be many patient groups that would not be considered appropriate candidates for transplant because their predicted outcomes would not be optimal. Modeling both of these systems we see an egalitarian system would give all an equal opportunity for a transplant but the overall graft outcome would suffer. In the utilitarian system, we would enjoy the best graft outcome but at the cost of many lives, lives that could have enjoyed a reasonable graft outcome rather than facing death on the waitlist.

In order to address this issue, we looked at new data regarding HLA matching, allocation and outcomes in renal transplantation. Several very interesting and convincing pieces of data were presented by the SRTR (Scientific Registry for Renal Transplantation: http://www.ustransplant.org). Data were presented that evaluated the impact of each individual locus (A, B and DR) on renal transplant outcome. The data clearly showed there were minimal effects of A-locus and B-locus mismatching, but mismatching for 1 or 2 DR antigens showed a significant decrease in graft survival (12 percent for 1-DR MM and 22 percent for 2-DR MM). These data were compelling and led to the belief the current B-DR point allocation system should be revised. With this in mind, data were also presented that showed the impact on allocation and outcome (graft survival) if; 1) all HLA matching were dropped (excluding mandatory 6-ag match) or, 2) if only B-locus matching was dropped. The modeling showed if all HLA matching were dropped there would be an approximate 11percent increase in allocation of organs to minorities. The down side was a predicted 8percent increase in graft loss. By contrast, dropping only B-locus matching produced an 8 percent increase in allocation to minorities with only a predicted 2 percent increase in graft losses. Based on all the data, it was suggested that points should only be awarded for DR-matching. This idea will be presented to UNOS for upcoming debate.

Remember this conference was only to investigate issues and make recommendations to UNOS. Any proposal will still have to pass through the complete UNOS process including public comments. Interestingly, an additional proposal was put forth to consider actually subtracting points (i.e.; awarding negative points) for very poor matches; e.g., 5 and 6 antigen mismatches. This idea was quite interesting and may also be suggested to UNOS.

Lastly, the group on managing the waitlist provided one proposal that was important to ASHI members. This proposal had to do with managing patients on the waitlist and how often it is necessary to screen samples for alloantibody. This working group came up with the following recommendation that will be presented to UNOS: new patients should be screened for a period of at least 3 consecutive months to establish a baseline PRA. Subsequent to this initial evaluation period, patients could be screened on a quarterly basis. It would be up to the individual laboratory/transplant program to provide data supporting their choice of interval for antibody screening. Note, however, this group strongly supported the current practice of obtaining monthly samples from patients who are

"active" on the waitlist so crossmatching could be performed using the most current sera. Of additional importance with respect to PRA testing was the belief that HLA laboratories should provide support for antibody specificity analysis. Both the list management group as well as the group that discussed access, strongly felt knowing unacceptable antigens is most useful if sensitized patients are going to be considered for transplants that are not 6-antigen matched. Our clinical colleagues challenged the HLA community to provide such testing with the goal of being able to predict negative crossmatches for such sensitized patients.

The Waitlist Conference is just one example where individuals from ASHI were involved in potential policy changes. Another area was the recent proposed changes to the UNOS policies that were sent out for public comments. I hope that all of you took the time to submit comments. The Scientific Affairs committee, Chaired by Dr. Peter Nickerson, put together a very nice set of responses for ASHI and these were submitted. I include them below for your review.

Douglas A. Heiney,
Director Department of Membership Services
and Policy Development
UNOS
1100 Boulders Pkwy
Suite 500
P.O. Box 13770
Richmond, VA 23225-8770

Dear Mr. Heiney,

The American Society for Histocompatibility and Immunogenetics (ASHI) welcomes the opportunity to provide comments on the recent proposals put forth by UNOS (UNOS Policy Proposals, March 15, 2002). We hope that the following comments will be helpful in the subsequent discussions.

==========

Response to Proposal 2:

In this proposal, data is presented that demonstrates no tangible benefit from HLA matching for pancreas alone or pancreas after kidney transplantation. The recommendation therefore is to "remove priority for HLA matching from the system for pancreas allocation, with the exception of priority assigned for zero antigen mismatches between donor and patient." The data to back up this recommendation is a study based on 484 cadaveric pancreas only transplants and 866 cadaveric pancreas-after-kidney transplants. The study period was from Jan 1995 to March 2001, with average follow-up to 3 years.

Based on direct inspection of the available data the recommendation appears to be sound; however, there are a few comments we think should be made:

1] The study is based on a relatively small number of transplants and as such, while the recommendation may be adopted, it should not apriori prevent ongoing analyses of this issue when a larger data set is available. Thus, UNOS should adopt a mechanism to revisit this issue on an annual basis.

2] The immunosuppression that has been used in pancreas transplantation to date has been quite intense. In the current era of immunosuppression there is a move towards minimizing immunosuppressive protocols in order to avoid drug-associated toxicities/side-effects. This being the case, the relative importance of HLA matching (or lack thereof) may become evident as global immunosuppression is reduced. Thus, this fact also supports the recommendation put forth in #1 above.

3] It is strongly recommended the HLA information of the donors and recipients continue to be collected prospectively. Such data elements should include: the HLA type of both donor and recipient, Peak and Current PRA values as well as any crossmatching results. This information will be necessary for subsequent data analyses but more importantly, would be essential if a given patient would require re-transplantation.

Response to Proposal 7:

In proposal #6, recommendations are made to collect various data in order to generate a pre-transplant risk profile for patients awaiting heart transplant. Included in this model are recipients Peak and Current PRA values. In contrast, Proposal 7, which recommends collection of similar data in order to generate a pre-transplant risk profile for patients awaiting lung transplant, does not include Peak and Current PRA values.

There have been recent publications in the Transplantation literature that anti-donor HLA antibodies in lung transplant can be associated with a detrimental outcome.

{[Hu. Immunol. 62(suppl.1):S25, 2001.]; [Transplantation 71(5):649-651, 2001]; J. Thoracic & Cardiovascular Surgery 119(1)39-45, 2000]; [Am. J. Respiratory and Critical Care Medicine 163(2)437-442, 2001]; [Transplantation 68(10):1542-1546, 1999]}

We would suggest UNOS collect pre-transplant PRA information for patients awaiting lung transplant so this data can be incorporated into the risk assessment.

==========

Response to Proposal 8:

This is a proposal that was put forward by the UNOS Histocompatibility Committee making the argument Bw4 and Bw6 typing should be mandated for all prospective kidney and pancreas donors and recipients. Most typing centers are already performing this testing, collecting this data and should not pose any undue financial or technical hardship on the laboratories. ASHI supports this proposal.

Thank you for the opportunity to comment on these proposals.

Sincerely,

Robert A. Bray, Ph.D., Diplomate (ABHI)

President, American Society for Histocompatibility and Immunogenetics

I would like to take just a moment and speak briefly about ASHI's financial position. An audit of ASHI finances has been completed. As of the date of this letter we have not yet seen the final numbers but will have them soon. The financial situation of ASHI, while still sound, is of concern to all on council. The costs of doing business have risen significantly and we have incurred some additional expenses as part of our transition to the new management company. Also, as many of you, know the performance of the stock market has been less than expected. Much of ASHI's assets are in Certificates of Deposit and Mutual Funds, which, although somewhat protected, are still at the mercy of the market. Thus, part of our concern for ASHI finances has been the reduced ASHI equity as a result of the market fluctuations. Nonetheless, ASHI remains strong. A significant part of our strategic plan is to keep ASHI solvent. This was one of the reasons for considering a change in management companies. I hope by the time of my next letter I will be able to give you a more detailed analysis of our financial situation.

ASHI has finalized the specifics of our enhanced proficiency-testing program. No longer a partner with CAP, ASHI is providing a new and restructured PT program. I encourage each of you to read the announcement in this issue of the *Quarterly* and contact the ASHI office directly if you have any questions. We are hopeful our new PT program will better meet the needs of our members as well as serve as an outstanding source for scientific information.

The planning for the 28th Annual ASHI Meeting is wrapping up. This year's program promises to be quite exciting. I hope you each will take the time to join your colleagues in Nashville, TN.

The last item I would like to touch on briefly is the recent and tragic passing of J. Marilyn MacQueen. Marilyn was one of the founding individuals of SEOPF, then AACHT and ultimately ASHI. Although recently retired from the laboratory, Marilyn's contributions to our field have been quite numerous. I direct you to the article in this issue written by those who knew her best. She will be missed.

In closing, I would like to say during the first few months of 2002, ASHI council has been quite busy. Although we have accomplished much, there is still a significant amount of work that lies ahead. In order for ASHI to be successful we will need the input and assistance of all ASHI members. We look forward to your participation.

EDITORIAL

A National Conference to Analyze the Wait List for Kidney Transplantation

Steven K. Takemoto, Clinical Science Editor

On March 4-5 2002, the National Kidney Foundation along with AST and ASTS sponsored a meeting to examine the implications of the expanding list of patients waiting for renal transplants and to formulate recommendations to address past policy that has resulted in an accumulation of highly sensitized and ethnic minorities on the waiting list. Four working groups were formulated to address specific issues: (1) Access to the list, (2) Assigning priority on the wait list, (3) Management of the list and (4) Expanded criteria for donor kidneys. In this article, I will summarize the findings of the conference with particular attention to issues that will affect the Histocompatibility community.

The overall theme of the meeting was certain ethnic groups are disproportionately denied access to transplantation. The first work group addressing access to the list found although all patients on dialysis could benefit from a transplant and Medicare will cover the cost of the transplant, the level of education, socioeconomic status and the area where one lives all influence the probability a dialysis patient will be referred to a transplant program. They found many patients were not adequately informed of their entitlement to receive a transplant and efforts to educate these patients are warranted. Also, many generic insurance policies that provide a cut rate to employers do not fully cover the costs of the transplant procedure. A lobbying effort is recommended to counter this current trend.

The issues debated in the second work group most directly affected our community. The question on everyone's mind was how to resolve the perception the allocation system is unfair to minorities because of HLA typing. Since the chairs of the group strived to form a consensus, our representatives were able to preserve the current policy of national sharing for zero mismatched transplants. There was actually widespread support to continue this policy and the benefit to those fortunate enough to receive HLA matched kidneys was not questioned. The most vocal criticism was the disadvantage to OPOs composed mainly of ethnic minorities. They are dealt a double disadvantage when imported matched kidneys, usually to white recipients, are paid back with a second kidney.

The Scientific Registry in Michigan presented the most provocative result affecting our group. In a cohort of patients transplanted since 1995, one or two B locus mismatches only increased the relative risk of graft loss by 7 percent over zero B locus mismatches. This difference was very modest when compared to the 12 percent increase for 1 DR and 22 percent increased risk for 2 DR mismatches. This result provided the impetus to eliminate B locus mismatches from the local sharing algorithm, which would increase the allocation to minorities by 11 percent. The cohort used for this seminal study excluded pre-1995 data, the justification for the exclusion being differential outcome of recent transplants. Past literature suggests the impact of class II mismatches occurs earlier than class I mismatches so it remains to be shown that a long-term benefit for class I mismatches will not be borne out in the MMF era. The change to eliminate B level matching must be subjected to public scrutiny before it can become national policy.

Other recommended changes to national policy were 1) only allow donor O kidneys to be allocated to zero mismatched B or O but not A recipients since B is prevalent in Blacks and A is so frequent. 2) Expand priority given to zero mismatched patients with PRA >30 percent (not just >80 percent)when multiple HLA matched patients are identified on the national list. 3) Initiate waiting time when a patient begins chronic dialysis to eliminate both the advantage due to early access to the list and the penalty for inactivation while recovering from health related setbacks.

One recommendation that may change the way we perform antibody screens was also proposed in the second working group. One method for improving equity is to increase the size of the allocation unit. In order to do so, one must be fairly confident that the crossmatch for the intended recipient would be negative. The transplant community sought ways to identify patients likely to have a positive crossmatch when knowledge of the donor HLA typing becomes available. The solution offered by our colleagues was more extensive pretransplant testing to specifically and accurately identify those HLA antigens reactive to recipient sera and listing of these antigens as unacceptable antigens in the OPTN data record for waiting recipients. This effort may greatly reduce the number of crossmatch tests performed for each donor and may therefore streamline the allocation process. Now may be a good time to consider adopting screening strategies utilizing state of the art single antigen reagents since transplant programs and HHS will be receptive to our efforts to improve allocation efficiency.

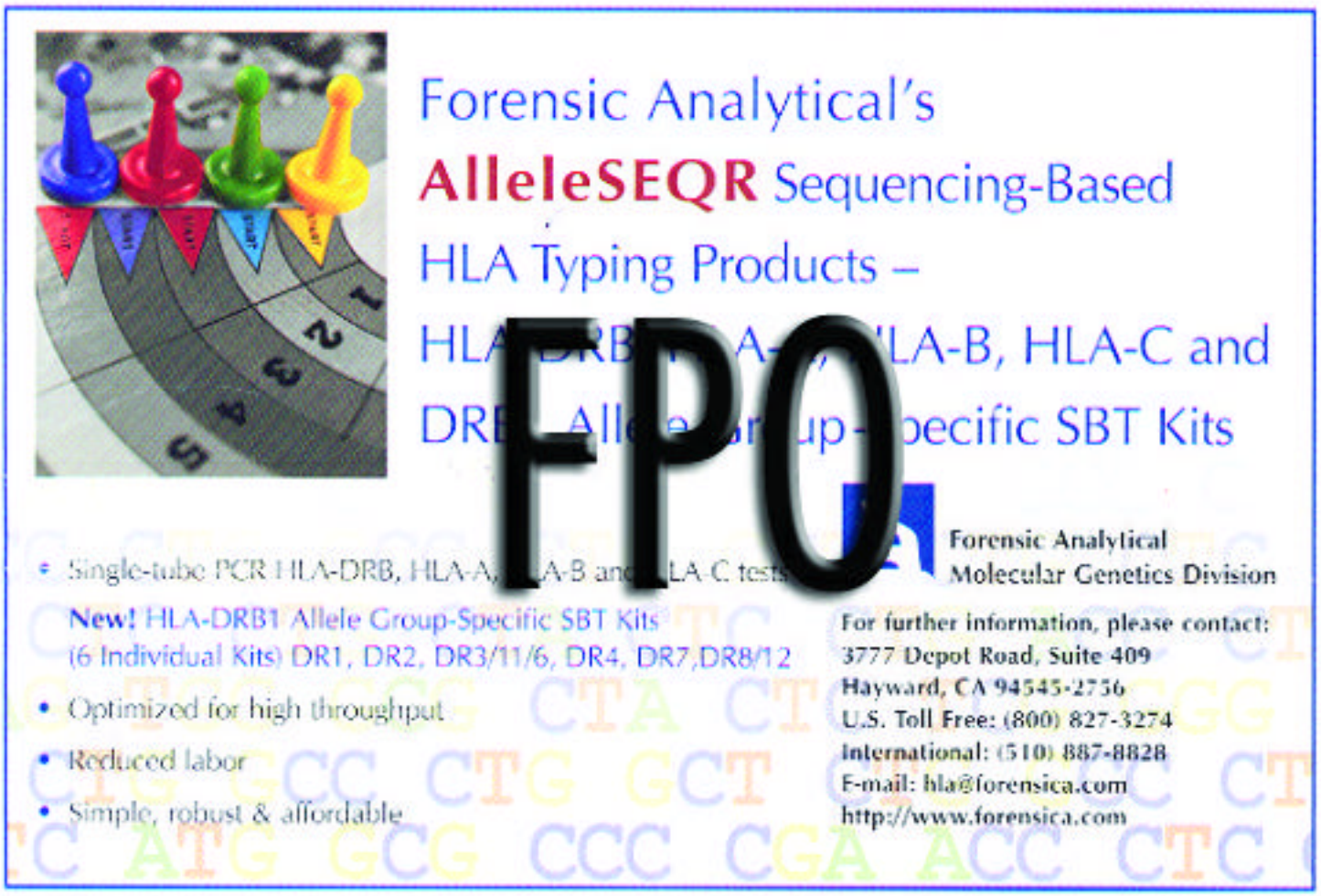
Because of a lack of demonstrable improvement in outcome, one OPO has given up their efforts to use CREG matching to prioritize the waiting list. It was clear that CREG matching was more equitable than prioritization by B and DR mismatches, but the group decided instead to recommend priority be given to 0 DR mismatched candidates. Plans have been made, however, to examine whether CREG matching can improve distribution of the list so sensitized recipients likely to have a negative crossmatch are ordered to the top. An examination of alternative CREG models will also be made to determine if more complex systems yield an improved outcome.

Waiting lists at many centers have grown to the point that it is difficult to keep tabs on all of the patients. Some centers are following as many as 1,400 transplant candidates. The strategy

employed by many centers is to attempt to identify those patients likely to receive a transplant in the near future. If allocation were based only on waiting time, only those patients waiting for a long time would be candidates. HLA matching, on the other hand, provides the possibility every patient on the list might be the next candidate. Consequently, every patient must be ready for transplant. The charge of the third group was to devise ways to manage the large lists. Suggestions were made to reduce the priority for national sharing to allow more efficient allocation of staff resources. A compromise was reached when our colleagues illustrated the probability of a patient to receive a zero mismatched graft could be calculated through haplotype frequencies. Another recommendation from this group was to devise rational antibody screening protocols, especially for patients with no demonstrable antibody, to minimize costs of maintaining patients on the list for periods of time that have already extended beyond 5 years in many areas and are expected to approach 10 years by 2010.

The fourth work group dealt with issues involving expanded criteria for donor kidneys. They seek to implant these organs as quickly as possible. To accommodate this goal, it would be necessary to implement protocols to complete workup and typing as quickly as possible. Efforts should be made to obtain samples as quickly as possible and minimize the time to complete the crossmatch procedure. Time limits were recommended for transplant centers to accept these kidneys. A recommendation was made to limit national sharing to recipients willing to accept this expanded kidney. If sensitized recipients are to be given the opportunity to benefit from these kidneys, it would be imperative for us to streamline the crossmatch process to quickly identify those recipients with a negative crossmatch.

It is gratifying to know many of our colleagues in the transplant community appreciate the work we do to streamline the procedure of placing cadaveric organs and improve the quality of life for transplant recipients. There are many challenges ahead to further improve the service we provide.



ASHI Version

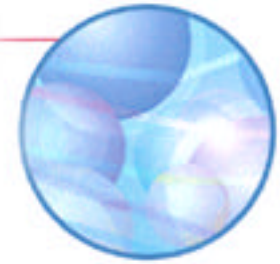
Single Antigen Screening Is Here.

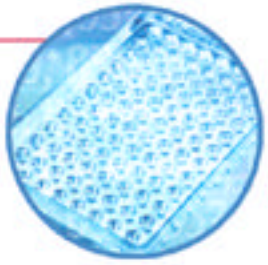
Uncover hidden specificities masked in high PRA sera. Using your choice of methodology, flow cytometry or ELISA, our new products, **FlowPRA™ Single Antigen** and **Lambda Antigen Tray™ (LAT) Single Antigen**, provide unique tools for the evaluation of high PRA patients.

Features and Benefits

- Feature single antigens per well or per bead
- Uncover the masked specificities by broad epitopes
- Determine antigens that are not reactive with patients
- [illegible] as strategic [illegible] specific results

FPO





[illegible] is [illegible] single Class I antigen wells and 6 control wells [illegible] Terasaki tray. The tray features a well-balanced panel of specificities and all wells feature a single antigen. In addition, common antigens are represented at least twice on the panel. The multi-use test can be used as a routine antibody detection screening test or as a supplementary test for high PRA patients.

Coming Soon! LABScreen™ Single Antigen

For further information on both FlowPRA™ and LAT™ Single Antigen tests, or any of One Lambda's quality HLA products, contact your One Lambda representative or visit our web site.

One Lambda, Inc.

21001 Kittridge St., Canoga Park, CA 91303-2801, USA
TEL: 818/702-0042 800/822-8824 (except greater L.A. area)
FAX: 818/702-6904 800/992-2111 (U.S. and Canada only)
INTERNATIONAL: Contact your local distributor
www.onelambda.com

For In Vitro Diagnostic Use.

© Copyright 2002, One Lambda, Inc. All rights reserved. ™LAT and FlowPRA are a trademarks of One Lambda Inc.

HLA and Platelet Transfusion Outcome

Ena Wang, Francesco M. Marincola and Harvey G. Klein

Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland 20892

**Address correspondence to Dr. F. M. Marincola at Department of Transfusion Medicine, Clinical Center, Building 10, Room 1C-711, 10 Center Drive MSC 1502, Bethesda, MD 20892-1502, U.S.A.*

Tel: (301) 496-9702

Fax: (301) 594-1981

E-mail: marincola@nih.gov

Almost 4 million platelet transfusions are administered annually to acutely or chronically thrombocytopenic patients in the United States [1]. Approximately 10 percent of these platelet transfusions have limited clinical success with minimal or no increase in post-transfusion platelet count. Severe, persistent "refractoriness" is believed to be due predominantly to previous immune sensitization of the host against allo-antigens carried by the donor platelets [2]. Indeed, platelet donation by unrelated donors to thrombocytopenic individuals may be envisioned as a limited form of transplantation, where the transplanted tissue has a short but definite expectation of survival in the recipient with no possibility of self-perpetuation. Thus, the end-point of transfusion is an almost mathematical increment in circulating platelet number that is, in turn, influenced by several factors, predominant among which is their relationship with the host immune system. Thus, the study of platelet immune refractoriness presents a somewhat simplified model of allo-transplantation in which only a few aspects of immune-rejection are evaluated at one time. In this model, humoral and cellular responses against allogeneic cells are analyzed directly and independently of other factors that might affect survival of solid organ transplants independent of host's reactivity. These include impaired vascularization due to degenerative changes of the vascular supply or poor transplant take due to technical failures or sub-optimal viability of the transplanted tissues (i.e. duration of ischemia, etc).

Platelet persistence in patients with thrombocytopenia who have received a transfusion follows a relatively straightforward algorithm whereby the number of platelets at a given time point will depend upon their expected half life minus their accelerated destruction by the pre-sensitized host. This destruction is believed to be due primarily (although not exclusively [3]) to the presence of unmatched HLA class I molecules on the surface of the donor's platelets toward which the recipient displays immune reactivity after previous allo-sensitization. HLA alleles determine the tissue compatibility that is necessary for compatibility of transplanted tissue. Indeed, graft survival in solid organ transplantation is influenced by the number of HLA mismatches between donor and recipient [4-6]. For instance, when the influence of HLA compatibility on organ transplant survival was analyzed in more than 150,000 recipients, a strong positive effect was noted on graft survival by complete matching of HLA class I and class II loci. In addition, correction of serological typing errors using DNA-based molecular typing resulted in improved matching effects 4. Similarly, it has long been known that in large part immune refractoriness in platelet recipients is due to anti-HLA reactivity by the host immune system and most strategies are focused on the reduction of donor/recipient mismatch [7,8]. On clinical grounds, several strategies have been proposed to manage the platelets for "immune refractory" patients such as reducing the density of HLA molecules on the surface of donated platelets or augmenting donor/recipient compatibility by supporting donor/recipient selection with various cross-matching or typing methods. Modifying the patients' immune response with plasmapheresis and intravenous immune globulin administration has proven unsatisfactory.

Decreasing the Density of HLA Molecules on the Surface of Platelets (Acid Elution)

In vitro elution of peptide/HLA complexes using mildly acidic components (i.e. acidic acid) can strongly reduce the expression of HLA molecules on the surface of any cell or corpuscle including platelets. With the same treatment, functionally relevant platelet-specific glycoproteins are preserved. Thus, studies have suggested treating platelets with mild acids to decrease the level of HLA incompatible molecules expressed on their surface and decreasing, therefore, the chances of allogeneic interactions between donor and recipient [9]. This strategy has maintained platelet counts in a limited number of refractory patients. While this strategy seems promising, standardization of the acid elution method and validation of the technique remains under investigation. In addition, it is not clear whether HLA class I expression might be reconstituted in vivo after transfusion when "empty" HLA heavy chains are exposed to endogenous peptides.

Increasing Donor/Recipient Compatibility

a) *Crossmatch-based selection.* Crossmatch compatibility has been used for the identification of candidate platelet donors [10]. This method has the theoretic advantage of directly predicting recipient/donor compatibility. However, crossmatching requires re-evaluation of each individual donor/recipient pair. A disadvantage of

crossmatching is platelets have a very short half life. Therefore, for patients who require long-term platelet transfusion, frequent crossmatching needs to be performed. The great diversity of platelet antigens and their variable expression has limited the usefulness of commercial test kits. However, in a recent retrospective study, it appeared the administration of crossmatch compatible platelets significantly improved the survival of platelets in refractory patients independent of their allo-immunization status [10].

b) *Identification of anti-HLA antibodies in the serum of recipients.* In most cases, patients who are refractory to platelet transfusion as a result of allo-immunization are given HLA-matched or crossmatched platelets. However, compatible platelet donors are not always readily available. Thus, as an alternative, selection of donor platelets could be performed by excluding platelet donations from donors expressing HLA molecules for which the refractory recipient has shown evidence of pre-sensitization. This is based on the identification of pre-existing HLA allo-reactive antibodies in the serum of the recipient (antibody specificity prediction method or ASP)[11]. ASP is effective in selectively excluding HLA alleles for which pre-sensitization has occurred. This strategy, therefore, bypasses the need for a more extensive match. This, in turn, has the important advantage of broadening the availability of compatible platelet components. Evidence from solid organ transplantation suggests that, although HLA matching is beneficial regardless of the status of pre-sensitization, matching is most effective in recipients displaying cytotoxic antibodies suggesting pre-sensitization strongly affects graft survival [4]. Various strategies have been reported for identification of allo-antibodies based on complement-mediated cytotoxicity or fluorimetric testing [12]. Exclusion of HLA phenotypes based on evidence of humoral pre-sensitization is, however, relatively inaccurate and the method is subject to misinterpretation. In addition, although "partial matching" may prevent platelet destruction due to pre-existing allo-antibodies, it does not prevent the development of novel allo-reactivities in the recipient due to the additional exposure to HLA mismatched platelet components. Finally, since a role of T cells in inducing platelet destruction has not been thoroughly investigated, it is possible antibody-based screening may underestimate the presence of an ongoing cellular immune response.

c) *Heightening donor/recipient histocompatibility.* A common practice in the past has been to administer to patients who are refractory to random-donor platelets HLA-matched or "semi-matched" platelet pheresis components [3;10;11;13]. This strategy is based on the characterization of donor and recipient HLA phenotype and selection of pairs associated with the lowest level of mismatch. Generally, HLA mismatches have been identified using serological techniques (also called "antigen mismatches"). More recently, HLA mis-matching information derived through DNA-based typing has been accrued (also termed "allele mismatches). Whether both degrees of polymorphism are relevant in the context of allo-immunization remains to be elucidated since the significance of the finer resolution provided by DNA-based typing has been questioned in the context of bone marrow transplantation [14]. In this review, we will pay special attention to the role that high-resolution HLA typing may play in improving the outcome of platelet transfusion therapy.

Impact of High-Resolution HLA Typing

Independent of the technique used, optimization of donor/recipient selection and interpretation of clinical results will ultimately depend upon the level of resolution provided by the HLA typing method used for recipient/donor selection. In most centers, platelet selection for patients who are refractory to random-donor platelets is largely based on the pre-identification of potential donors from a consistent pool of HLA typed donors. Interestingly, provision of "HLA matched" donor platelets does not always correlate with enhanced platelet survival. While such refractoriness may be related to non-immunologic factors or platelet-specific antigen, it may well be caused by unrecognized HLA mismatches between donor and recipient related to the low-resolution methods adopted in the past for HLA typing.

Conceptually, the usefulness of conventional serologic assays for identification of HLA antigen mismatches has been limited by the availability of allele-specific sera. In addition, such assays do not discriminate between allelic sub-types within each serologic family and, therefore, cannot identify allele mismatches. As antibodies identify structural differences on the surface of HLA molecules, protein structure differences caused by single or limited nucleotide polymorphisms within the peptide-binding groove away from the surface of the HLA heavy chain are not detectable by antibody-based techniques. In recent years, HLA typing has dramatically increased in resolution through the utilization of polymerase chain reaction (PCR) targeting genomic sequences specific for individual HLA alleles within classic serologic families (allelic typing) [15-17]. Differences detectable by molecular typings are of functional significance as they determine the specificity and affinity of peptide binding [18;19] and T cell recognition of self or allogeneic cells [20;21]. Thus, molecular typing allows theoretically unlimited discrimination among HLA alleles and should have, in principle, the advantage over serological typing of allowing a better definition of donor/recipient compatibility which should in turn result in improved clinical outcome. Since, however, it is not presently known what role T cells play in immune rejection of platelets in addition to antibody-mediated mechanisms. It is not known how fine molecular differences in the internal structure of HLA variants may influence platelet survival. In fact, the usefulness of high-resolution typing has been recently questioned in the context of hematopoietic-cell transplantation [14;22] and little is known about its applicability in the context of platelet transfusion. In fact, it is possible that allele-mismatches detectable only at DNA level may be less immunogenic than antigen mismatches and, therefore, they may be associated with lower risk of platelet transfusion failure. In the context of hematopoietic cell transplantation, antigen mismatches seem to confer a higher risk of graft failure while a single HLA allele mismatch did not increase such risk [22]. The biochemical reasons for such disparity, however, did not appear clearly in the study, since graft failure was also noted in patients with antigen mismatch in which the mismatch occurred predominantly in the peptide-binding region not directly exposed to antibody or TCR receptor.

Through PCR $_{15}$ new alleles have been identified and confirmed by direct sequencing [23]. Sequence-specific, labeled synthetic oligonucleotides have been used as probes for hybridization to genomic DNA amplicons with the purpose of detecting single nucleotide polymorphisms or other insertion/deletion polymorphisms [24]. PCR-sequence-specific oligonucleotide probe (PCR-SSOP) hybridization utilizes short PCR-derived fragments of DNA designed to encompass sequences of genomic DNA specific for most and/or theoretically all known allelic variants [17]. Sequence-specific primer (SSP)-PCR is based on a similar principle that takes advantage of known segments of sequence diversity in the genome of various HLA alleles to design allele-specific primers [25]. This method based on two, rather than one oligonucleotide sequence allows more flexibility than SSOP but also is susceptible to more intricate interpretation due to the overlap between various allelic sequences. Both methods allow typing to a level of resolution that can be tailored, according to specific clinical needs, to identify any known HLA allele. They have, however, are two major limitations. First a disproportionate amount of workload is necessary to obtain this information due to the ever-growing list of HLA alleles. Thus, these techniques are suitable for the identification of HLA restriction elements required for the enrollment of patients in HLA-associated treatments such as epitope-specific immunizations [26]. However, global matching for the purpose of transplantation or transfusion cannot be limited to a few super-families of HLA alleles although, for platelet donation ,HLA matching could be limited to HLA class I -A and -B loci since platelets do not express HLA class II and express irrelevant amounts of HLA-C molecules [27]. A further limitation of PCR-based typing is that only known HLA alleles can be identified based on knowledge of the variant-specific sequence for the design of primers/probes. If the primers/probes do not encompass a segment of genomic DNA where a new polymorphism is located, polymorphism will be missed and mis-typing will occur. Occasionally, new alleles have been identified using PCR-based methods, but in those cases, the polymorphic site coincided with a genomic sequence encompassed by the primer set used for typing[28].

The limitations of PCR-based typing could be overcome by sequence-based typing (SBT) that directly identifies the nucleotide sequences of each allele allowing exact assignment independent of the presence of known or unknown polymorphic segments [29]. In addition, this technique has the advantage of providing direct information about each individual's phenotype. In theory, therefore, SBT should be more time saving and cost effective for large-scale histocompatibility testing. However, although SBT has been applied for research and clinical use, its utilization has been limited by cost of equipment and reagents, the high level of expertise required and time necessary for interpretation of typing results. Yet we have routinely used SBT to confirm patients' suitability for accrual in immunization protocols restricted to one HLA class I and/or class II allele [29-31]. We are presently starting a prospective evaluation of patient/donor selection based on the definition of a donor pool typed by SBT. Available information will be used to identify appropriate matches for SBT typed recipients. It has been suggested recently the type and location of mismatched amino acid residues may be important in determining the clinical permissibility of hematopoietic-cell transplantation [22]. SBT-based selection of donor-recipient pairs will allow the development of a grading system to assess the level of histocompatibility based on number of mismatches. In addition the number of amino acid residues involved in individual mismatches and their location in the HLA molecule could be defined. This in turn will allow predictions about peptide binding, T cell receptor interaction or exposure to allo-antibodies for individual alleles.

Summary

Survival of allogeneic heterologous platelets represents a simplified model of allo-transplantation often markedly dependent on the level of recipient immune reactivity against donor HLA antigens/alleles. Analysis of donor-recipient histocompatibility may, therefore, shed light on some of the intricacies of transplant survival. As SBT might gradually replace other, less-definitive, typing methods it will be important to prospectively test the feasibility and usefulness of SBT-based HLA typing for platelet donors / recipient matching. The following points should be addressed:

1) Utilization of complementary pre-sensitization screening tools such as anti-HLA antibody identification or cross matching.

2) Development of a grading system for HLA matching between donors and recipients based on homozygosity of recipient, number of mismatches stratified according to serological or molecular detectability and their molecular location [22].

3) Stratification of mismatch according to HLA class I loci (A, B and C)

4) Utilization of the grading system for optimization of donor selection

5) Correlation of clinical information with grade of HLA class I matching.

6) Determination of the usefulness of high-resolution histocompatibility assessment for the treatment of pre-existing transplant refractoriness (allo-immunized recipient) and the prevention of refractoriness in naïve non-refractory individuals.

References

1. 1999. National Blood Resource Center. Comprehensive report on blood collection and transfusion in the United States in 1999. AABB Press (Bethesda, MD) 1:13-23.

2. 1997. The trial to reduce alloimmunization to platelets Study Group. Leukocyte selection and ultraviolet B irradiation to prevent alloimmunization and refractoriness to platelet transfusion. N Engl J Med 26:1861-1869.

3. Kekomaki, S., L. Volin, P. Koinstinen, E. Koivunen, S. Koskimies, T. Ruutu, T. Timonen, and R. Kekomaki. 1998. Successful treatment of platelet transfusion refractoriness: the use of platelet transfusions matched for both human leucocyte antigens (HLA) and human platelet alloantigens (HPA) in alloimmunized patients with leukemia. Eur J Haematol 60:112-118.

4. Opelz, G., T. Wujciak, B. Dohler, S. Scherer, and J. Mytilineos. 1999. HLA compatibility and organ transplant survival. Collaborative transplant study. Rev Immunogenetics 1:334-342.

5. Opelz, G. and T. Wujciak. 1994. The influence of HLA compatibility on graft survival after heart transplantation.The Collaborative Transplant Study. N Engl J Med 330:816-819.

6. Takemoto, S. K., P. I. Terasaki, D. W. Gjertson, and J. M. Cecka. 2000. Twelve years' experience with national sharing of HLA-matched cadaveric kidneys for transplantation. N Engl J Med 343:1078-1084.

7. Yankee, R. A., F. C. Grumet, and G. N. Rogentine. 1969. The selection of compatible platelets donors for refractory patients by lymphocyte HLA-typing. N Engl J Med 281:1208-1215.

8. Duquesnoy, R. J., D. J. Filip, G. E. Ridey, A. A. Rumm, and R. H. Aster. 1997. Successful transfusion of platelets "mismatched" for HLA antigens for alloimmunized, thrombocytopenic patients. Am J Hematol 2:219-225.

9. Novotny, V. M., T. W. Huizinga, R. van Doorn, E. Briet, and A. Brand. 1996. HLA class I-eluted platelets as an alternative to HLA-matched platelets. Transfusion 36:388-391.

10. Gelb, A. B. and A. D. Leavitt. 1997. Crossmatch-compatible platelets improve correct count increments in patients who are refractory to randomly selected platelets. Transfusion 37:624-630.

11. Petz, L. D., G. Garratty, L. Calhoun, B. D. Clark, I. P. Terasaki, C. Grensen, J. A. Gornbein, E. M. Landaw, R. Smith, and J. M. Cecka. 2000. Selecting donors of platelets for refractory patients on the basis of HLA antibody specificity. Transfusion 40:1446-1456.

12. Moses, L. A., D. Stroncek, K. M. Cipolone, and F. M. Marincola. 1999. Detection of HLA antibodies using flow cytometry and latex beads coated with HLA antigens. Transfusion 40:861-866.

13. Ornstein, D. L., K. L. Mortara, M. B. Smith, D. W. Ririe, P. J. Shaughnessy, D. J. Bickford, and B. L. Kissack. 2001. Treatment of sever thrombocytopenia in alloimmunized, transfusion-refractory patients. Mil Med 166:269-274.

14. Rubenstein, P. 2002. HLA matching for bone marrow transplantation - How much is enough? N Engl J Med 345:1842-1843.

15. Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol 51:263-273.

16. Player, M. A., K. C. Barracchini, T. B. Simonis, L. Rivoltini, F. Arienti, C. Castelli, A. Mazzocchi, F. Belli, and F. M. Marincola. 1996. Differences in frequency distribution of HLA-A2 sub-types between American and Italian Caucasian melanoma patients: relevance for epitope specific vaccination protocols. J Immunother 19:357-363.

17. Shaffer, A. L., J. A. Falk-Wade, and V. Tortelli. 1992. HLA-DRw52-associated DRB1 alleles: identification using polymerase chain reaction-amplified DNA, sequence-specific oligonucleotide probes and chemiluminescent detection system. Tissue Antigens 39:84-90.

18. Parker, K. C., M. A. Bednarek, L. K. Hull, U. Utz, B. Cunningham, H. J. Zweerink, W. E. Biddison, and J. E. Coligan. 1992. Sequence motifs important for peptide binding to the human MHC class I molecule, HLA-A2. J Immunol 149:3580-3587.

19. Rotzschke, O., K. Falk, S. Stevanovic, G. Jung, and H. G. Rammensee. 1992. Peptide motifs of closely related HLA class I molecules encompass substantial differences. Eur J Immunol 22:2453-2456.

20. Sette, A., A. Vitiello, B. Reherman, P. Fowler, R. Nayersina, W. M. Kast, C. J. Melief, C. Oseroff, L. Yuan, J. Ruppert, and et al. 1994. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. J Immunol 153:5586-5592.

21. Rivoltini, L., D. J. Loftus, K. Barracchini, F. Arienti, A. Mazzocchi, W. E. Biddison, M. L. Salgaller, E. Appella, G. Parmiani, and F. M. Marincola. 1996. Binding and presentation of peptides derived from melanoma antigens MART-1 and gp100 by HLA-A2 subtypes: implications for peptide-based immunotherapy. J.Immunol. 156:3882-3891.

22. Petersdorf, E. W., J. A. Hansen, P. J. Martin, A. Woolfrey, M. Malkki, T. oley, B. orer, E. ckelson, A. ith, and C. Anasetti. 2001. Major-histcompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. N Engl J Med 345:1794-1800.

23. Rodriguez, S. G., C. L. Crevling, N. Steiner, and C. K. Hurley. 1996. Identification of a new allele, DRB1*1204, during routine PCR-SSOP typing of National Marrow Donor Program Volunteers. Tissue Antigens 48:221-223.

24. Wallace, R. B., M. J. Johnson, T. Hirose, T. Miyake, E. H. Kawashima, and K. Iyakura. 1981. The use of synthetic oligonucleotide as hybridization probes. II. Hybridization of oligonucleotides of mixed sequence to rabbit b-globin DNA. Nucleic Acids Res 9:879-894.

25. Bunce, M., C. M. O'Neill, M. C. Barnardo, P. Krausa, M. J. Browning, P. J. Morris, and K. I. Welsh. 1995. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens. 46:355-367.

26. Cormier, J. N., M. L. Salgaller, T. Prevette, K. C. Barracchini, L. Rivoltini, N. P. Restifo, S. A. Rosenberg, and F. M. Marincola. 1997. Enhancement of cellular immunity in melanoma patients immunized with a peptide from MART-1/Melan A [see comments]. Cancer J.Sci.Am. 3:37-44.

27. Datema, G., S. Stein, C. Eijsink, A. Mulder, F. H. Class, and I. I. Doxiadis. 2000. HLA-C expression on platelets: studies with an HLA-Cw1-specific human monoclonal antibody. Vox Sang 79:108-111.

28. Bettinotti, M., C. J. Kim, K.-H. Lee, M. Roden, J. N. Cormier, M. C. Panelli, K. C. Parker, and F. M. Marincola. 1998. Stringent allele/epitope requirements for MART-1/Melan A immunodominance: implications for peptide-based immunotherapy. J Immunol. 161:877-889.

29. Adams, S. D., P. Krausa, M. McGinnis, T. B. Simonis, J. Stein, and F. M. Marincola. 2001. Practicality of high-throughput HLA sequencing based typing. ASHI Quarterly 25:54-57.

30. Adams, S. D., K. C. Barracchini, T. B. Simonis, D. Stroncek, and F. M. Marincola. 2001. High throughput HLA sequence-based typing utilizing the ABI prism 3700 analyzer. Tumori 87:s41-s44.

31. McGinnis, M., J. B. Stein, S. D. Adams, F. M. Marincola, and P. Krausa. 2001. Implementing high throughput HLA SBT on a 96-capillary DNA sequencer. Hum.Immunol. s146:7.4.

HLAMatchmaker: A Molecularly Based Algorithm For Histocompatibility Determination

Rene J. Duquesnoy, Ph.D.
University of Pittsburgh Medical Center

Introduction

In organ transplantation, donor-recipient histocompatibility is generally determined by counting the number of mismatched antigens in the donor's HLA-A, B, DR phenotype. Many studies have shown that zero-HLA antigen mismatched transplants have the highest success rates and these findings have led to the allocation policy of mandatory sharing of zero-mismatched cadaver kidneys. Another method for assessing donor-recipient compatibility considers public epitopes that are shared by HLA antigens belonging to so-called cross-reacting antigen groups (CREGs). Several studies have suggested CREG matching is associated with graft survivals that are better or comparable to those allocated on HLA antigen matching, but others have not found a beneficial effect of CREG matching. The controversy about CREG matching is due in part to the difficulty to define the exact spectrum of public epitopes on all HLA antigens. The CREG matching system in UNOS utilizes ten CREGs but the actual number of public epitopes is considerably higher. Amino acid sequence information on HLA antigens has led to the identification of polymorphic residues that correspond to many public epitopes and private HLA antigens.

HLA matching is important for alloimmunized patients and serum screening may yield information about specific antibodies to public epitopes and the HLA antigens in the corresponding CREGs are considered unacceptable mismatches. Serum reactivity patterns are usually analyzed by 2x2 table statistics but this correlation analysis does not permit a meaningful interpretation if the Panel Reactive Antibody (PRA) activity exceeds 85 percent. Often enough, many highly sensitized patients remain on the waiting list with little prospect of a transplant because the probability of finding a zero antigen mismatch is very low.

HLAMatchmaker is a computer algorithm especially designed to identify compatible HLA antigens for highly allosensitized patients [1-3]. Donor-recipient HLA compatibility is assessed at the structural level by intralocus and interlocus comparisons of polymorphic amino acid triplet sequences in alloantibody-accessible positions of HLA molecules namely, the α-helices and β-loops of the protein chain structure. The residues in the strands of the β-pleated sheets of the peptide-binding groove are excluded from this matching algorithm because they cannot make direct contact with alloantibodies.

Each triplet is designated by its amino acid composition around a given position in the amino acid sequence. Amino acid residues are marked with the standard letter code; an uppercase letter corresponds to the residue in the numbered position whereas the lowercase letters describe the nearest neighboring residues. For instance, the triplet a65rNm represents an asparagine residue (N) in position 65 with arginine (r) in position 64 and methionine (m) in position 66 of the HLA-A chain. Many triplets are marked with one or two residues because their neighboring residues are the same on all HLA Class I chains and they are therefore not shown. For instance, b12aM represents an alanine residue in position 11 and a methionine residue in position 12 on HLA-B chains. The triplet b41T has a threonine in position 41 and the two neighboring monomorphic residues are not shown.

HLA Matching at the Triplet Level

HLAMatchmaker applies two principles: (1) each HLA antigen represents a distinct string of polymorphic triplets as potential immunogens that can induce specific alloantibodies and, (2) patients cannot make alloantibodies against triplets on their own HLA molecules [2]. The algorithm assesses donor-recipient compatibility through intralocus and interlocus comparisons, and determines what triplets on mismatched HLA molecules are different or shared between donor and patient. This analysis considers each donor HLA antigen mismatch towards the entire HLA-A, B, C phenotype of the recipient.

As an example let us consider a kidney donor who types as HLA-A2, A32; B8, B55; Cw3, Cw6 There are two transplant candidates: patient A types as HLA-A2, A30; B18, B27; Cw2, Cw4 and patient B types as HLA-A2, A31; B42, B53; Cw2, Cw7. This donor is a three-HLA-A, B antigen mismatch for both patients. Which patient is the best recipient? HLAMatchmaker determines if triplets on a donor antigen are found in the same sequence position on any of the patient's HLA antigens. For instance, the donor's HLA-B8 represents a mismatched antigen by conventional criteria but at the structural level, HLA-B8 has six mismatched triplets for the HLA-A2, A30; B18, B27; Cw2, Cw4 but has no mismatched triplets for HLA-A2, A31; B42, B53; Cw2, Cw7 (Table 1). Altogether, this donor's HLA antigens have a total of 14 different mismatched triplets for patient A (A32: 3 triplets, B8: 6 triplets, B55: 3 triplets, Cw3: 2 triplets and Cw6: 1 triplet), but for patient B, all of them are zero-triplet mismatches. Thus, at the structural level, this donor appears fully compatible to patient B but not to patient A. This matching algorithm is at the humoral immune level and is particularly useful for highly alloimmunized patients.

Application of HLAMatchmaker after Serum Analysis of Highly Sensitized Patients

The number of HLA antigens with zero or few triplet mismatches depends on the HLA phenotype of the patient. For some patients is it difficult to find HLA antigens matched at the

Table 1. Examples of HLA compatibility determination at the amino acid triplet level.

Patient 1	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	138
A2	F	sV	R	gR	A	Me	G	Ge	rKv	aHs	H	Vd	gTl	IRg	A	S	W	K	R	T
A30	S	sV	R	gS	A	Me	R	Qe	rNv	aQs	D	Vd	gTl	IRg	A	S	G	N	R	T
B18	H	sV	R	GR	A	Te	G	Rn	qIs	tNt	Y	Es	rNl	IRg	A	P	G	N	s	T
B27	H	sV	R	gR	A	Ee	G	Re	qIc	aKa	D	Ed	rTl	lLr	A	P	G	N	S	T
Cw2	Y	aV	R	sR	A	Ge	G	Re	qKy	rQa	D	Vn	rNl	IRg	A	P	G	N	R	T
Cw4	S	sV	W	gR	A	Ge	G	Re	qKy	rQa	aD	Vn	rNl	IRg	D	P	G	N	R	T
Donor HLA-B8	D	aM	R	gR	A	Ee	G	Rn	qIf	tNt	D	Es	rNl	IRg	A	P	G	N	R	T

Patient 1(contd)	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
A2	T	tKh	W	aAh	aHv	L	A	T	Ew	Y	Et	Q	A	K	Av	A	S	A	V	Q
A30	I	tQr	W	aAr	aRw	L	A	T	Ew	Y	Et	Q	P	K	Pi	A	G	A	V	E
B18	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	P	K	Pi	A	G	A	V	E
B27	I	tQr	W	aAr	aRv	L	A	E*	Ew	Y	Et	Q	P	K	Pi	A	G	A	V	E
Cw2	I	tQr	W	aAr	aRe	W	A	E*	Ew	Y	Et	Q	Eh	K	Pv	A	G	A	V	E
Cw4	I	tQr	W	aAr	aRe	R	A	T	Ew	Y	Et	Q	Eh	K	Pv	A	G	A	V	E
Donor HLA-B8	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	P	K	Pi	A	G	A	V	E

Patient 2	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	138
A2	F	sV	R	gR	A	Me	G	Ge	rKv	aHs	H	Vd	gTL	IRg	A	S	W	K	R	T
A31	T	sV	R	gR	A	Me	R	Qe	rNv	aHs	iD	Vd	gTL	IRg	A	S	G	N	R	T
B42	Y	sV	R	gR	A	Ee	G	Rn	qIy	aQa	D	Es	rNl	IRg	A	P	G	N	R	M
B53	Y	aM	R	gR	A	Te	G	Rn	qIf	tNt	Y	En	rIa	aLr	A	P	G	N	S	M
Cw2	Y	aV	R	sR	A	Ge	G	Re	qKy	rQa	D	Vn	rKl	IRg	A	P	G	N	R	T
Cw7	D	aV	R	gR	A	Ge	G	Re	qNy	rQa	aD	Vs	rNl	IRg	D	P	G	N	R	T
Donor HLA-B8	D	aM	R	gR	A	Ee	G	Rn	qIf	tNt	D	Es	rNl	IRg	A	P	G	N	R	M

Patient 2 (contd)	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
A2	T	tKh	W	aAh	aHv	L	A	T	Ew	Y	Et	Q	dA	K	Av	A	S	A	V	Q
A31	I	tQr	W	aAr	aRv	L	A	T	Ew	Y	Et	Q	dP	K	Av	A	S	S	V	Q
B42	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	dP	K	Pi	A	G	A	V	E
B53	I	tQr	W	aAr	aRv	L	A	L	Ew	Y	Et	Q	dP	K	Pv	A	G	A	V	E
Cw2	I	tQr	W	aAr	aRe	W	A	E	Ew	Y	Et	Q	eH	K	Pv	A	G	A	V	E
Cw7	I	tQr	L	aAr	aRa	L	A	T	Ew	Y	Et	Q	eP	K	Pl	A	G	A	V	Eq
Donor HLA-B8	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	dP	K	Pi	A	G	A	V	E

Analysis was based on the following molecular types. Patient 1: HLA-A*0201, A*3001; B*1801,B*2705; C*0202,C*0401. Patient 2: HLA-A*0201,A*3101; B*4201,B*5301; C*0202, C*0701. Donor: HLA- A*0201,A*3201; B*0801,B*5501; C*0302,C*0602

triplet level. As an example, patient 3 typed as HLA-A3, A11; B18, B62, Cw7, - and was highly sensitized with a PRA of 96 percent. HLAMatchmaker analysis showed B75 as a zero-triplet mismatch and B72 and B76 as one-triplet mismatches. These antigens have very low frequencies in our donor population and it appeared unlikely that HLAMatchmaker would increase compatible donor availability for this patient. Our PRA analysis showed that 48 of 50 HLA-typed panel cells reacted with patient's serum. Two panel cells gave consistently negative reactions. From their phenotypes, HLA-A3, A26; B62, - and HLA-A11, -; B18, B51, it should be noted that these negative cells shared HLA antigens with the patient. These negative cells expressed two mismatched antigens: A26 and B51. Table 2 shows that A26 has eight mismatched triplets and B51 has five mismatched triplets. Because patient's antibodies did not react with A26 or B51, these triplets were considered acceptable mismatches. After entering A26 and B51 as "negative antigens" in HLAMatchmaker we identified eight additional HLA antigens that were zero/acceptable triplet mismatches namely, A25, A32, A66, A74, B35, B52, B53 and B77. This example illustrates how a serum analysis combined with HLAMatchmaker can be useful in strategies to increase donor availability for highly sensitized patients.

Triplet Matching and Kidney Transplant Survival

Recent studies have been conducted to determine how class I HLA matching at the triplet level affects kidney transplant outcome. We have analyzed two multi-center databases of zero-HLA-DR mismatched kidneys transplanted in 1987-1999. One dealt with 31879 primary allografts registered by US transplant centers in the United Network for Organ Sharing (UNOS) database and the other consisted of 15,872 transplants in the Eurotransplant program.

The results show that HLA-A, B mismatched kidneys that were compatible at the triplet level exhibited almost identical graft survival rates as the zero HLA-A, B antigen mismatches defined by conventional criteria [4]. This beneficial effect of triplet matching was seen for both non-sensitized and sensitized patients and also for white and non-white patients. The practical implication of this finding is the possible use of the HLAMatchmaker algorithm to increase the number of well-matched transplants [5]. Furthermore, triplet matching may especially benefit sensitized patients and non-white transplant candidates for whom it is difficult to find donors with good matches.

Table 2. Identification of acceptable triplet mismatches from negatively reacting panel cells mismatched for HLA-A26 and HLA-B51.

Patient 3	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	138
A3	F	sV	R	gR	A	Me	G	Qe	rNv	aQs	D	Vd	gTL	lRg	A	S	G	N	R	T
A11	Y	sV	R	gR	A	Me	G	Qe	rNv	aQs	D	Vd	gTL	lRg	D	P	G	N	R	T
B18	H	sV	R	gR	A	Te	G	Rn	qIs	tNt	Y	Es	rNl	lRg	A	P	G	N	S	M
B62	Y	aM	R	gR	A	Ma	G	Re	qIs	tNt	Y	Es	rNl	lRg	A	P	G	N	S	M
A26 Mismatch	Y	sV	R	gR	A	Me	G	Rn	rNv	**aHs**	D	**An**	gTL	lRg	D	P	G	N	R	T
B51 Mismatch	Y	aM	R	gR	A	Te	G	Rn	**qIf**	tNt	Y	**En**	**rIa**	**aLr**	A	P	G	N	S	M

Patient 3 (contd)	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
A03	I	tKr	W	aAh	aHe	L	A	dT	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
A11	I	tKr	W	aAh	aHa	Q	A	R	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
B18	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	dP	K	Pi	A	G	A	V	E
B62	I	tQr	W	aAr	aRe	W	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
A26 Mismatch	I	tQr	W	**tAh**	aHe	W	A	R	Ew	Y	Et	Q	**dA**	K	**Av**	A	**S**	**S**	V	**Q**
B51 Mismatch	I	tQr	W	aAr	aRe	L	A	L	Ew	H	Et	Q	dP	K	**Pv**	A	G	A	V	E

Analysis was base on the following molecular types: Patient 3: HLA-A*0301,A*1101; B*1801,B*1501. Mismatched antigens: HLA-A*2601 and HLA-B*5101.

Why do the zero-triplet and few-triplet mismatched transplants appear so successful? Designed originally for highly sensitized patients [1, 2], the HLAMatchmaker algorithm considers only triplets that are in antibody-accessible positions of the HLA molecular structure. HLA-specific antibodies play a major role in graft rejection [6] and matching at the humoral immune level, i.e. for epitopes recognized by such antibodies can be expected to improve graft survival. HLA compatibility must also consider cellular immune mechanisms of graft rejection such as class I HLA specific cytotoxic T-lymphocytes and indirect allorecognition of processed donor class I HLA antigens presented by recipient CD4 T-cells. Although HLA matching at the cellular immune level must use different structural criteria (they are currently not defined), this humoral immunity-based matching strategy permits the identification of triplet matches among the zero-HLA-DR mismatches.

Why does HLAMatchmaker use Triplets?

The question can be answered by examining how antigens react with specific alloantibodies. Three-dimensional structures of different antigen-antibody complexes have shown that up to six hypervariable loops (or Complementarity Determining Regions, CDRs) of the antibody binding sites make contact with protein antigen. The contact area between antibody and antigen is about 700 to 800 square Angstroms and this is similar to the size of the HLA molecular surface seen from above the peptide-binding region and the alpha helices. . An epitope on a protein antigen contains a few critical residues that provide dominant contributions to the binding energy with one of the CDRs. There are also many contact residues that interact with other CDRs to increase the overall association of the immune complex, but they are not necessarily required for antibody specificity. Thus, in the case of an HLA-specific antibody, it seems likely that one CDR plays a primary role in the specific binding with a polymorphic triplet whereas the other CDRs interact with other sites on the HLA molecule; such sites may have monomorphic and/or polymorphic residues.

This concept may increase our understanding of the reactivity of complement-dependent, lymphocytotoxic alloantibodies against HLA and why their detection is so often technique-dependent. As an example, let us examine the reactivity of antibodies against A25 and A26. More than twenty-five years ago, we published two articles on what was considered at that time, two new splits of HLA-A10 [7, 8]. Our serum screening efforts had identified several monospecific anti-A25 and anti-A26 sera that were used for typing purposes by many laboratories worldwide. Absorption/elution studies showed that these sera exhibited CYNAP (i.e. the Cytotoxicity-Negative, Adsorption-Positive phenomenon) towards the other split of A10 whereas anti-A10 antibodies reacted with both A25 and A26 cells by complement-dependent lymphocytotoxicity. How are these findings explained?

Since we had HLA typing information about antibody producers and immunizers, it was possible to conduct an HLAMatchmaker analysis to determine what triplets were different on the immunizing HLA antigen (Table 3). In all cases, the antibodies appeared specific for 150tAh and this triplet is found exclusively on HLA-A10 molecules, including A25 and A26. In the case of the A25-specific typing sera Jun and Schu, the antibody producer had been exposed to 150tAh and five other triplets on the immunizing A25 antigen. This antibody showed lymphocytoxicity reactivity towards A25 but CYNAP activity towards A26. It seems likely one CDR of this antibody reacted with 150tAh but this reaction by itself was not strong enough for the antibody to bind C1q, the first component of complement necessary for initiating the process

Table 3. Triplet matching analysis of the serological reactivity of antibodies induced by HLA-A25 and HLA-A26

Serum	HLA-type Ab Producer	Ag	Reaction	Unshared Triplets on Antigen
Jun (anti-A25)	A1,A3;B7,B8	A25*	CYT+	80rIa 82aLr 150tAh 156W 183A 193Av
		A26	CYNAP	150tAh 156W 183A 193Av
Schu (anti-A25)	A2,A68;B37,-	A25*	CYT+	76Es 80rIa 82aLr 90D 150tAh 163R
		A26	CYNAP	76An 90D 150tAh 163R
Mich (anti-A26)	A3,A23; B7,B13	A26*	CYT+	76An 90D 150tAh 156W 163R 183A 193Av
		A25	CYNAP	90D 150tAh 156W 163R 183A 193Av
Tyl (anti-A26)	A2,A11; B13,B62	A26*	CYT+	62Rn 76An 150tAh
		A25	CYNAP	62Rn 80rIa 82aLr 150tAh
Sand (anti-A10)	A1,A24; B7,B44	A25*	CYT+	66eNv 150tAh 156W 183A 193Av
		A26	CYT+	66eNv 150tAh 156W 183A 193Av
Elli (anti-A10)	A1,A32; B13, B14	A26*	CYT+	150tAh 156W
		A25	CYT+	150tAh 156W

* Immunizing HLA antigen

leading to lymphocytotoxicity. Apparently, a second CDR of this antibody must react with another part of the antigen so the overall binding strength becomes sufficient for C1q activation. The unshared triplets that are present on A25 but not A26 appear to be the most likely recognition sites for the second CDR. Underlined in Table 1, they are 80rIa and 82aLr for antibody Jun and 76Es, 80rIa and 82aLr for antibody Schu. It should be noted the Jun and Schu antibodies do not see these triplets as specific epitopes because they do not react with 150tAh-negative cells that express 76Es, 80rIa or 82aLr.

Similar explanations can be offered for the reactivity of Mich and Tyl antibodies that exhibit lymphocytotoxicity towards A26 but CYNAP towards A25. In this case, the 76An triplet seems necessary for complement-dependent lymphocytotoxicity (Table 3). This analysis included also two sera that were specific for A10, i.e. A25+A26. The Sand antibodies were induced by A25 and the Elli antibodies were induced by A26. These antibodies showed lymphocytotoxicity against both A25 and A26 and HLAMatchmaker analysis showed no differences in unshared triplets between A25 and A26. Apparently, the second CDR needed for C1q binding and complement activation must have reacted with a triplet shared between A25 and A26.

In conclusion, these findings may provide some insight about the reactivity of alloantibodies with HLA antigens. One triplet presented by the immunizing antigen reacts with one CDR that apparently conveys the specificity of the antibody. Such antibody can be expected to react other antigens that express the same triplet, in other words a cross-reacting group of antigens. The remaining CDRs of the antibody must interact with other triplets originally present on the immunizing HLA molecule to increase the energy release during the formation of the antigen-antibody complex so C1q binding and subsequent complement activation and lymphocytotoxicity can take place. Cross-reacting antigens must have such triplets if they give positive reactions in direct complement-dependent lymphocytotoxicity. Absence of such triplets will diminish the binding energy release between antibody and the cross-reacting antigen as manifested by a negative lymphocytoxicity reaction although binding such as by ELISA testing might still be seen. The application of anti-human globulin (AHG) would be a useful source of additional binding energy for the activation of the complement cascade leading to lymphocytotoxicity.

References:

1. Duquesnoy, R.J., HLAMatchmaker: A Molecularly Based Donor Selection Algorithm For Highly Alloimmunized Patients. Transplantation Proceedings, 2001. 33: p. 493-497.
2. Duquesnoy, R.J., HLAMatchmaker: A Molecularly Based Algorithm For Histocompatibility Determination. I. Description of the Algorithm. Human Immunology, 2002. In Press.
3. Duquesnoy, R.J. and M. Marrari, HLAMatchmaker: A Molecularly Based Algorithm For Histocompatibility Determination. II. Verification of the Algorithm and Determination of the Relative Immunogenicity of Amino Acid Triplet-Defined Epitopes. Human Immunology, 2002. In Press.
4. Duquesnoy, R.J., S. Takemoto, P. De Lange, I.I.N. Doxiadis, G.M.T. Schreuder, and F.H.J. Claas, HLAMatchmaker: A Molecularly Based Algorithm For Histocompatibility Determination III. Effect of matching at the HLA-A,B amino acid triplet level on kidney transplant survival. Transplantation, 2002. Submitted.
5. Duquesnoy, R., J. Howe, M.D. Witvliet, I.I.N. Doxiadis, and F.H.J. Claas, HLAMatchmaker: A Molecularly Based Algorithm For Histocompatibility Determination. IV. Determination of Chances of Finding Donors with Zero/Acceptable Triplet Mismatches for Highly Sensitized Patients. Transplantation, 2002. Submitted.
6. McKenna, R.M., S. Takemoto, and P.I. Terasaki, Anti-HLA antibodies after solid organ transplantation. Transplantation, 2000. 69: p. 319-326.
7. Duquesnoy, R.J. and T.E. Schindler, Serological analysis of the HLA-A10 complex. Tissue Antigens, 1976. 72: p. 65-73.
8. Hackbarth, S.A. and R.J. Duquesnoy, Serologic analysis of the HLA-A10 complex II. Absorption studies on Aw25,Aw26 and Aw34. Transpl. Proc., 1977. 9(Suppl. 1): p. 43-45,.

Polymorphism and Antigenicity of HLA-MICA

Peter Stastny, M.D

MICA and MICB are genes in the MHC class I region, which are polymorphic, do not appear to present peptides, are not associated with beta$_2$-microglobulin and have a selective tissue distribution, mostly in epithelial cells, endothelial cells and fibroblasts. The term MICA, which stands for "MHC class I related chain A" was first used by Bahram and coworkers[1] in 1994. This gene was described independently by another group of workers, who called it PERB 11.1[2] in the same year. The WHO Nomenclature Committee for Factors of the HLA System opted for the name HLA-MICA. In the 2000 report 51 alleles of MICA were recognized and were given numbers from MICA*001 through MICA*046. Several new alleles have been described since then.

Because of the location of MICA, in the HLA class I region, close to HLA-B and only about 46 kb centrometric to it, linkage disequilibrium between alleles of MICA and of HLA-B has been observed[2]. With more than 50 alleles described to date, one can say the polymorphism is quite remarkable. Blasczyk and coworkers[3] have recently analyzed the nucleotide diversity of MICA and MICB and compared it with that of HLA-B. It is of interest the majority of the nucleotide substitutions are expressed. Therefore, the polymorphism probably did not arise by fixation of random events, but was driven by selective pressure perhaps from infectious agents. Other characteristics are that the polymorphism is not concentrated around the peptide binding groove, as is the case in HLA-A, B and C. Also, most of the polymorphic sites only display two possible substitutions. The functional significance of MICA is only beginning to be understood. It has been shown to bind to NKG2D, a stimulating receptor of NK cells, as well as CD8+ T cells and gamma-delta T cells. The possible significance of MICA in defense against pathogens is actively being investigated. This role is the most likely cause of the polymorphism.

Our laboratory has been interested in another aspect of HLA-MICA, namely its antigenicity and its capacity to induce immune responses due to allogeneic differences, as may be the case in transplantation [4].

Initially, we investigated antibody production in rabbits immunized with MICA peptides[5]. Production of polyclonal antibodies was highly successful and rabbit sera were used in Western blot experiments to explore the tissue distribution of MIC proteins. Most of these rabbit sera reacted only with denatured products of MICA or MICB. However, serum from rabbit 622 was found to recognize native MICA and could be used in flow cytometry experiments. Subsequently, we transfected the MICA gene into Chinese hamster ovary (CHO) cells and used these CHO cells, expressing abundant MICA in the cell membrane, to immunize mice for production of monoclonal antibodies. The mice developed high titer antibodies and B cell clones making antibodies specific to MICA were selected after screening by ELISA and flow cytometry.

Recombinant MICA, containing the α1, α2 and α3 domains, was prepared in E.coli and was used to assay patient sera by ELISA4. Recombinant MICA were produced for several of the more frequent alleles, estimating that together they represented 70-80 percent of the MICA alleles found in a random normal Caucasian population. To our surprise, it was discovered many organ transplant recipients developed serum antibodies that reacted with the recombinant MICA alleles in the ELISA procedure. Composite results for a group of kidney, heart and lung recipients are shown in Table I. Overall, about 25 percent of the patients showed reactivity with r-MICA and 31 percent of the sera were found to be positive.

Sequencing of the MICA alleles, or typing by SSOP, was used to determine relationships, if any, between the specificity of serum antibodies and the MICA phenotypes of recipients and donors. In most cases, antibodies against MICA reacted with alleles other than those of the recipient. In some cases, the specificity of antibodies detected corresponded to MICA alleles found in the corresponding donor.

The possibility that MICA may play a role in transplant rejection is based on the following observations, which will be explored in more detail below:

1. MICA antibodies kill cells in complement-dependent cytotoxicity;
2. absorption studies showed that many alloantisera contain anti-HLA class I and anti-MICA antibodies;
3. endothelial cells express MICA on their surface.

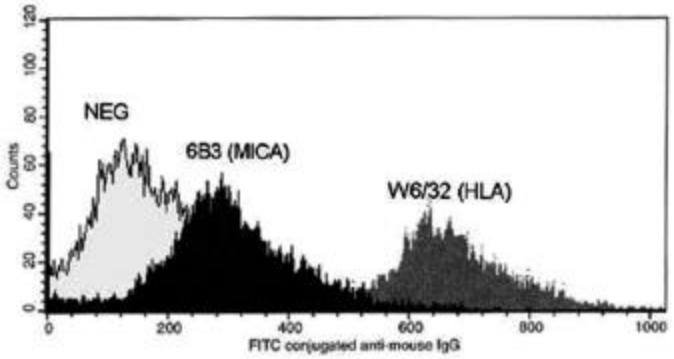


Figure 1

Human endothelial cells have MICA on their surface Endothelial cells were isolated from the vein of an umbilical cord and were cultured overnight. Monoclonal antibody 6B3 is specific for MICA. It does not react with MICB. Monoclonal antibody W6/32 recognizes HLA class I antigens.

HeLa is a human cell line that is known to be homozygous for MICA*008. Cytotoxicity experiments with monoclonal antibodies were performed by an antiglobulin technique, using goat anti-mouse Ig as the second antibody. The monoclonal antibody 3.2H3 specific for MICA, killed approximately 70

percent of the cells at a 1:100 dilution. W6/32, a monoclonal antibody against HLA class I, killed 100 percent of the cells. Another monoclonal antibody (2.3D4) known to recognize only denatured MICA, was used as a negative control and gave about 20 percent dead cells which was equal to the background with medium alone in this experiment. In other work, we used the human alloantisera CA and HA. After removal of HLA antibodies by repeated absorption with pooled human platelets, killing of HeLa cells was 75 percent and 70 percent, respectively. Subsequent absorption with recombinant MICA*008 removed all the cytotoxicity, thus confirming that the target antigen of the remaining antibodies was MICA[6].

These absorption experiments were very interesting. In flow cytometry experiments sera CA and HA, unabsorbed showed strong staining. After absorption with platelets to remove HLA antibodies staining was reduced but with both of these sera was still quite strong. After absorption with r-MICA*008, staining with these sera became weak. In cytotoxicity experiments performed in parallel, killing dropped from 100 percent to 70-75 percent after absorption with platelets. While the same sera absorbed first with platelets and then with r-MICA reduced the complement-dependent killing to the level of the negative controls (15 percent). These surprising results suggested that most of the alloreactivity of these two human sera was due to HLA antibodies and antibodies against MICA and that perhaps MICA alloantigens are more immunogenic than could have been previously suspected[6].

Table 1.

Development of anti-MICA antibodies in transplant recipients

Type of Transplant	Patients tested	Patients pos	Sera tested	Sera pos	
Kidney	56	12	205	62	
Heart	11	4	18	6	
Lung	5	2	7	4	
Total	72	18 (25%)	230	72 (31.3%)	

Another piece of evidence suggesting MICA could play a role in allograft rejection is the fact that these antigens are expressed on the surface of endothelial cells. For these experiments we isolated endothelial cells from human umbilical veins, cultured them overnight and performed staining with a MICA-specific monoclonal antibody and flow cytometry. As can be seen in Fig. 1, resting endothelial cells were clearly positive with the MICA-specific antibody 6B3, although the staining was not as intense as that for HLA class I obtained with W6/32. From previous work, in which flow cytometry was performed with monoclonal antibodies in multiple dilutions, we knew that at saturation, staining for HLA class I produced considerably greater fluorescence than staining for MICA, which is presumably a reflection of the higher concentration of HLA class I antigens compared to MICA on the cell surface6.

Altogether, our results suggest that MICA is both very polymorphic and highly antigenic. Alloantibodies can be readily detected in serum of some organ transplant recipients. They have also been found in subjects receiving transfusions and in women with multiple pregnancies. The ELISA method with r-MICA alleles provides a relatively easy way of determining sensitization against MICA. It is interesting that many patients who are sensitized against MICA do not have detectable antibodies against HLA class I. We have studied a number of patients who have low or negative PRAs, negative lymphocyte crossmatches against their donor by flow cytometry and who have expressed early severe, often irreversible, rejection of an allografted kidney. In some of these patients, we can detect the presence of MICA antibodies, which react with r-MICA alleles by the ELISA procedure.

Since antibodies against MICA can produce complement-dependent cytotoxicity and the MICA antigens are constitutively expressed on the surface of unstimulated endothelial cells, the possibility that these antigens may play a role in allograft failure in some recipients must be considered.

In conclusion, HLA-MICA genes are highly polymorphic with over 50 alleles having been described. The majority of the mutations are nonsynonymous and therefore not random. It appears likely MICA polymorphism is related in some way to resistance against pathogens. Immunization with MICA peptides, recombinant MICA and MICA transfectants has been uniformly successful in eliciting strong antibody responses. Alloantibodies against MICA have been found in many transplant recipients. Such sera are cytotoxic for cells expressing MICA on their surface and endothelial cells have been found to have MICA demonstrable by flow cytometry. The possible role of MICA in some forms of kidney allograft failure is being investigated.

References

1. Leelayuwat C, Townend DC, Degli-Esposti MA, Abraham LJ, Dawkins RL: A new polymorphic and multicopy MHC gene family related to nonmammalian class I. Immunogenetics 40(5):339, 1994.
2. Zhang Y, Lazaro AM, Lavingia B, Stastny P: Typing for all known MICA alleles by group-specific PCR and SSOP. Hum Immunol 62(6):620, 2001.
3. Elsner HA, Schroeder M, Blasczyk R: The nucleotide diversity of MICA and MICB suggests the effect of overdominant selection. Tissue Antigens 58(6):419, 2001.
4. Zwirner NW, Marcos CY, Mirbaha F, Zou Y, Stastny P: Identification of MICA as a new polymorphic alloantigen recognized by antibodies in sera of organ transplant recipients. Hum Immunol 61(9):917, 2000.
5. Zwirner NW, Fernandez-Vina MA, Stastny P: MICA, a new polymorphic HLA-related antigen, is expressed mainly by keratinocytes, endothelial cells, and monocytes. Immunogenetics 47(2):139, 1998.
6. Zou Y, Mirbaha F, Lazaro A, Zhang Y, Lavingia B, Stastny P: MICA is a target for complement-dependent cytotoxicity with mouse monoclonal antibodies and human alloantibodies. Hum Immunol 63(1):30, 2002.

ASHI Quarterly Continuing Education Quiz

Quiz Instructions: The multiple choice quiz below is provided as an alternative method of earning CE credit hours. Read each article then select the ONE best answer to each question. The questions are based solely on the content of the article. Answer all of the questions and send the quiz answer sheet and fee (if applicable) to the ASHI Executive Office before the processing date listed on the answer sheet. To be eligible for three CE credit hours, ALL questions must be answered, a passing score of 70 percent must be obtained and the answer sheet must be submitted with fee (if applicable) before the deadline. Quizzes may not be retaken, nor can ASHI staff respond to questions. Allow six weeks for processing following the submission deadline to receive return notification of your completion of the CE process. The CE credit will be dated when it is submitted for grading. That date will determine the CE cycle year.

Questions for "HLA and Platelet Transfusion Outcome"

After reading this article and taking the quiz the participants will demonstrate the ability to understand the role of HLA antigens in platelet transfusion outcome.

1. Platelet transfusion refractoriness is exclusively due to HLA class I mismatches.
   A. True
   B. False

2. Platelet refractoriness can be improved by the following strategy:
   A. Donor/recipient crossmatching
   B. Elution of HLA class I molecules from the platelet surface
   C. Identification of HLA reactive antibodies in the recipient
   D. HLA matching between donor and recipient.
   E. All of the above

3. Platelets do not express clinically relevant amounts of HLA-Cw molecules.
   A. True
   B. False

4. High resolution sequence-based typing may contribute in the future to recipient/donor selection because:
   A. Identified HLA alleles than cannot be identified by current serological techniques.
   B. Provides definitive typing of individual alleles independently of previous knowledge of their existence.
   C. Is relatively easier to perform than in the past because of the adoption of automated techniques
   D. All of the above

5. Platelet transfusion refractoriness occurs in approximately:
   A. 1% of cases
   B. 10% of cases
   C. 50% of cases
   D. it is extremely unusual

Questions for "Polymorphism and Antigenicity of HLA-MICA"

After reading this article and taking the quiz the participant wil demonstrate the ability to understand MICA genes, their possible significance and polymorphism.

6. MICA genes are:
   A. Non-polymorphic
   B. Associated with beta2-microglobulin
   C. In the MHC class I region
   D. Widely distributed in tissues

7. Polymorphism of MICA
   A. Arose from random events
   B. Caused by selective pressure
   C. Is concentrated around the peptide binding groove
   D. All of the above

8. MICA may play a role in transplant rejection because
   A. Endothelial cells express MICA on the surface
   B. Many alloantisera contain anti-MICA antibodies
   C. MICA antibodies kill cells in complement-dependent cytotoxicity
   D. All of the above

9. Anti-MICA antibodies were detected in
   A. 50% of all heart transplant recipients tested
   B. 25% of all transplant recipients tested
   C. 31% of all kidney transplant recipients tested
   D. None of the above

10. Alloantibodies to MICA are:
    A. Detected in serum of some organ transplant recipients
    B. Not found in people receiving transfusions
    C. Not found in women with multiple pregnancies
    D. Not demonstrated using the ELISA method

Questions for "Diversity of NK Cell Receptors and their HLA class I Ligands"

After reading this article and taking the quiz the participant will demonstrate the ability to understand the diversity of NK cell receptors and their functions.

11. The KIR are:
    A. Members of the immunoglobulin super gene family
    B. Encoded on human chromosome 12
    C. Lectin-like receptors
    D. Expressed by human CTL's

12. Long tail KIRs have:
    A. Activating function
    B. A charged residue in the transmembrane region
    C. ITIM
    D. ITAM

13. NKG2D:NKG2D homodimers recognize:
    A. MICA and MICB
    B. ULBP2
    C. MHC class I-like structure
    D. All of the above

14. KIR2DL1 binds to:
    A. HLA-A allotypes
    B. HLA-B allotypes
    C. HLA-C allotypes
    D. HLA-G allotypes

15. KIR "A-group haplotypes" are:
    A. Complicated, containing 9-12 genes
    B. Inhibitory for all major HLA class I specificities
    C. Activating type
    D. Simple, consisting of 7 non-functional genes

ASHI Quarterly Continuing Education Quiz — Answer Sheet

Please print clearly in black ballpoint pen.

First Name | M.I. | Last Name

Institution

Address

Address

City | State/Province | Zip Code/Postal Code

Country | ASHI Membership Number (**MUST** complete if requesting fee waiver)

Instructions: Mark your answers clearly by filling in the correct answer, like this ■, not like this ☒. Three contact hours earned with passing score (70%).

Please use black ballpoint pen.

1	A	B	C	D
2	A	B	C	D
3	A	B	C	D
4	A	B	C	D
5	A	B	C	D
6	A	B	C	D
7	A	B	C	D
8	A	B	C	D
9	A	B	C	D
10	A	B	C	D
11	A	B	C	D
12	A	B	C	D
13	A	B	C	D
14	A	B	C	D
15	A	B	C	D

For Internal Use Only

Date Received: _____

Amount Received: _____

Notification Mailed: _____

This original quiz answer sheet will not be graded, no CE credit will be awarded, and the processing fee will be forfeited unless postmarked by:

26 - 2 - 2002

Quiz Identification Number:

2002 - 020

ABHI Program Number:	CA Provider Number:	FL Provider Number:
2002-20	0085	JP480

☐ Processing Fee: **ASHI Member – no fee** **Nonmember – $30**

☐ Payment is due with submission of answer sheet. Make check or money order payable to ASHI (U.S. currency only). Do not send cash. No refund under any circumstances. Please allow six weeks following the submission deadline for processing.

☐ ASHI members and individuals paying by credit card may fax the answer sheet to (856) 439-9982.

Please check one:

☐ Enclosed is check #_____ (payable to ASHI)

☐ Charge to the following card:

☐ MasterCard (16 digits) ☐ Visa (13 or 16 digits)

Card Number_____ Exp. Date _____

Signature _____

Print Cardholder's Name _____

Mail to: ***ASHI Executive Office***
Len Morrissey
ASHI CE Quiz
17000 Commerce Parkway, Suite C
Mount Laurel, NJ 08054

Diversity of NK Cell Receptors and their HLA Class I Ligands

Raja Rajalingam

Departments of Structural Biology, Microbiology and Immunology
Sherman Fairchild Building, D150
Stanford University School of Medicine
Stanford, California 94305, USA.

Tel : 650-723-5469
Fax : 650-723-8464
E-mail: lingam@cmgm.stanford.edu

HLA class I mediate two distinct functions. The Major Histocompatibility Complex (MHC) is one of the most gene dense regions in the human genome[20, 33]. Two distinct families of genes in the MHC (class I and class II) encode highly polymorphic Human Leukocyte Antigens (HLA) that are involved in antigen presentation. Three classical class I genes (HLA-A, -B and -C) are normally expressed on the surface of most cells in the body and are recognized by two distinct cytolytic lymphocytes: cytotoxic T cells (CTL) and natural killer (NK) cells. Upon viral infection, class I molecules present viral peptides to the T cell receptors (TCR) of CTLs, which triggers a positive signal leading to lysis of the virally-infected target cells[10, 41] (Figure 1A). The natural killer (NK) cells express functionally two distinct sets of HLA class I-specific receptors: activating NK receptors and inhibitory NK receptors[15]. A fine balance between these two types of HLA class I-specific receptors controls NK cell function. Binding of HLA class I and specific inhibitory NK receptors generates a dominant inhibitory signal that neutralizes any positive signals in the NK cells, and thereby the self class I protects healthy cells from the NK lysis (Figure 1B). Some viral infections or tumor transformations downregulate the expression of certain HLA class I on the surface of infected cells to avoid the CTL response. In this context, no inhibitory signal is generated in the NK cells, which releases the lysis on infected cells[2,18] (Figure 1C). Further, binding of activating NK receptors with specific HLA class I or class I-like products on infected cells may boost the NK cell lysis. Thereby, CTLs and NK cells are the two complementary arms of cell-mediated cytotoxicity that govern response to infection.

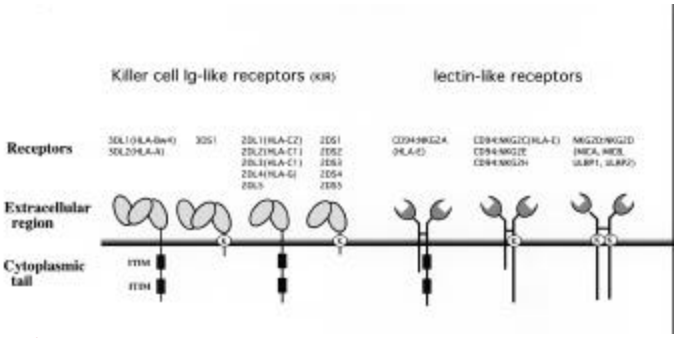


Figure 1
Cell mediated cytotoxicity. Interaction of HLA class I with their specific receptors govern the responses of T cells and NK cells. (A). HLA class I molecules mark the abnormal cells for cytotoxic T cell (CTL) lysis. (C). If such marking has failed due to virus-mediated class I downregulation, natural killer (NK) cells mediate the lysis. (B). Class I protects the healthy cells from NK lysis, i-NKR- inhibitory NK receptors, a-NKR- activating NK receptors.

NK cells express structurally and functionally distinct sets of HLA class I-specific receptors. Human NK cells express structurally two distinct families of MHC class I receptors: killer cell immunoglobulin-like receptors (KIR) and lectin-like receptors (Figure 2). The KIR are members of the immunoglobulin (Ig) super gene family and are encoded by a family of 14 polymorphic genes arranged in the Leukocyte Receptor Complex (LRC) on human chromosome 19.32,38. Based on the number of Ig-domains in the extracellular region, KIR receptors can be divided into either three Ig domains containing KIR (KIR3D) or two Ig domains containing KIR (KIR2D)(http://www.gene.ucl.ac.uk/nomenclature/genefamily/kir.html).[1,19] Based on the length of the cytoplasmic tail, they can be further grouped into either long (L) tail KIRs having inhibitory function, or short (S) tail KIRs with activating function. Long tail KIRs have one or two immunoreceptor tyrosine-based inhibitory motifs (ITIM) and transduce inhibitory signals. In contrast, the short tail KIRs have no ITIM motif but possess a charged residue in the transmembrane region that mediate association with DAP12. The DAP12 contains immunoreceptor tyrosine-based activation motif (ITAM) and transduces activating signals. [15]

The lectin-like receptors are encoded by a family of six conserved genes in the NK gene complex (NKC) on chromosome 12.[40] They are expressed as heterodimers (CD94:NKG2) or homodimers (NKG2D:NKG2D). Of these, only NKG2A has ITIM motifs in its cytoplasmic tail and transduces inhibitory signals. The rest of the receptors in this family have no ITIM but have a charged residue in their transmembrane region and bind to the positive signaling adaptor molecules, DAP12 and DAP10.

NK cell receptors have distinct MHC class I specificity. The CD94:NKG2 heterodimers are specific to HLA-E, a non-classical MHC class I. [4] The NKG2D:NKG2D homodimers recognize a variety of ligands having MHC class I-like structure. These include MHC class I-like chain (MICA and MICB), and human cytomegalovirus (HCMV) encoded UL16 binding

proteins (ULBP1, ULBP2, and ULBP3). The co-crystal structures of NKG2D:NKG2D/MICA [16] and NKG2D:NKG2D/ULBP3 [25] revealed that the NKG2D homodimers recognize multiple ligands by the induced-fit mechanism.

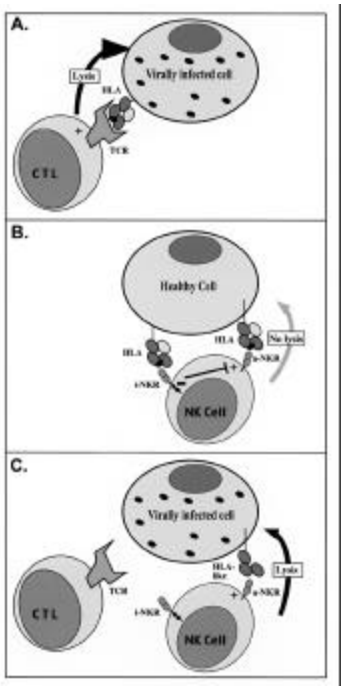


Figure 2
HLA class I-specific receptors of human NK cells. Human NK cells express two families of class I-specific receptors. Their structural diversity in the extracellular region is adapted to diverse HLA class I specificities. Diversity in the cytoplasmic tails are used for inhibitory and activating function. The specificities for individual NK receptors are given in parentheses. ITIM- immunoreceptor tyrosine-based inhibitory motifs.

The KIR receptors are specific to polymorphic classical class I HLA molecules. The co-crystal structures of HLA-Cw4/KIR2DL1 and HLA-Cw3/KIR2DL2 revealed a lock-and-key interaction.[3,8] The general topology of HLA/KIR binding is very similar to HLA/TCR binding. The footprint of TCR covers almost the entire surface of the antigen binding groove of HLA class I. In contrast, the KIR binding is restricted to one end of the peptide-binding groove. KIR interacts with both the α1 and α2 domain helices of class I, as well as with the carboxyl terminal portion of the bound peptide. Although both HLA and KIR are polymorphic, most of the residues in the HLA/KIR interface are conserved. The KIR2DL1, KIR2DL2 and KIR2DL3 are specific to HLA-C molecules.[5,21,37,39] A single amino acid residue in these KIR2Ds (position 44 in D1 domain), and a single amino acid in the HLA-C allotypes (position 80) determine their allotypic specificity. The KIR2DL1 has a methionine at position 44 and binds to HLA-C allotypes (Cw2, 4, 6, 15) having lysine at position 80. KIR2DL2 and KIR2DL3 have a lysine at position 44, and they bind to HLA-C allotypes having asparagine at position 80. The KIR3DL1 binds to HLA-B allotypes carrying Bw4 epitope. [11,17] KIR3DL2 binds to certain HLA-A (A3 and A11) allotypes[7,23], and KIR2DL4 binds to HLA-G.[24,26] The specificity of other KIR receptors are not known.

Individuals have distinct KIR types and distinct HLA types. Genomic plasticity and rapid evolution of the KIR region contribute to a unique haplotypic diversity in the number and type of KIR genes.[13,28,38] This produces a huge diversity in the number and combination of KIR genes an individual inherits.[34] Based on gene contents, the KIR haplotypes can be distinguished into two major groups. The "A-group haplotypes" are relatively simple, consisting ~7 functional genes. They include inhibitory KIR genes for all major HLA class I specificities, and have one or two activating KIRs (KIR2DS2 and/or KIR2DS4). The "B-group haplotypes" are complicated, and containing 9-12 genes. They are marked by KIR2DL5 gene[36], and most of the KIR genes in the B-group haplotypes are activating type. B-group haplotypes may not carry inhibitory KIR genes for some HLA specificities. Both groups of haplotypes conserve only three functional genes, and are considered to be the `framework' genes.

KIR genes are known to be polymorphic. [9,27,31] The allelic differences in the KIR genes are scattered throughout the entire coding regions.[9,27]This is in contrast to HLA allotypes, where the substitutions are primarily concentrated in the region forming the peptide-binding groove.[12,22] The combined diversity of haplotypes and allotypes distinguish individuals in the population.[29,30] Furthermore, alternative splicing of RNA produces additional diversity within some KIR sequences at the transcriptional level.[6] The NK cells of an individual express different numbers and combinations of KIR and lectin-like receptors that contribute to an additional complexity in the NK cell receptor repertoires.[35] NK cells may express 2-9 KIR receptors, which include at least one inhibitory receptor for the self class I. Variability in the gene content, allelic polymorphism, altered transcription and combinational expression of KIR diversifies the NK cells.

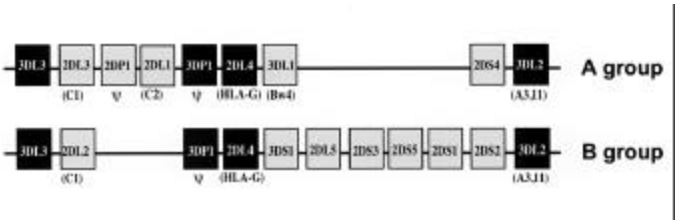


Figure 3
Genomic organization of two groups of KIR haplotypes. The class I specificities are shown in parentheses. C1- HLA-Cw1,3,7,8, C2- HLA-Cw2,4,6,15. Genes conserved in both groups of haplotypes are shown in dark boxes. y indicates pseudogenes.

Conclusion. The genes for HLA and KIR are polygenic and polymorphic and are located on different chromosomes. Their independent segregation and co-evolution complicate the number and type of NK receptor/HLA ligand pairs inherited in individuals. Since the NK receptors/HLA ligand pairs control the NK cell function, their status from "none to too many" may determine the clinical outcome of infection.

References

1. Andre, P., Biassoni, R., Colonna, M., Cosman, D., Lanier, L. L., Long, E. O., Lopez-Botet, M., Moretta, A., Moretta, L., Parham, P., et al. (2001). New nomenclature for MHC receptors, Nat Immunol 2, 661.
2. Biron, C. A., Nguyen, K. B., Pien, G. C., Cousens, L. P., and Salazar-Mather, T. P. (1999). Natural killer cells in antiviral defense: function and regulation by innate cytokines, Annu Rev Immunol 17, 189-220.

American Society for Histocompatibility and Immunogenetics

28th Annual Meeting

October 19 - 23, 2002

Opryland Hotel
Nashville, TN

Program Chair:
Dolly B. Tyan, PhD,
Diplomate, ABHI

American Society for Histocompatibility and Immunogenetics 28th Annual Meeting

October 19 - 23, 2002 • Opryland Hotel • 2800 Opryland Drive • Nashville, TN 37214
615.889.1000 • Fax: 615.871.5843

Schedule of Events

Saturday, October 19, 2002

1:00 PM • 8:00 PM	Registration
7:00 PM • 10:00 PM	Welcome Reception

Sunday, October 20, 2002

7:00 AM	Registration
8:00 AM • 10:00 AM	Opening Session with Keynote Speaker: *Peter Doherty, Nobel Laureate*
	Exhibit Hall Open and Posters
10:30 AM • 12:30 PM	Plenary 1: Proteomics
	a. Mass Spectrometry - *Peter Nelson*
	b. Protein Chips - *Emanual Petricoin*
	c. Clinical Proteomics - *Paul Herrmann*
2:00 PM • 4:00 PM	Plenary 2: New Technology
	a. Expression Profiling in SLE - *Tim Behrens*
	b. Autoantigen Microarrays - *William Robinson*
	c. Lympho Chip - *Cor Verweij*
	d. Real Time PCR - *Roy Swiger*
4:30 PM • 6:00 PM	Abstracts #1
	Abstracts #2
	Workshop #1 - Medium Density Arrays - *Randy Saiki*
	Workshop #2 - Advanced Flow Cytometry - *Robert Bray and Howard Gebel*
6:00 PM • 7:30 PM	Wine and Exhibitor/Poster Session #1
7:30 PM • 9:00 PM	Inspectors Reception and ARB International Reception

Monday, October 21, 2002

7:00 AM • 5:00 PM	Registration
8:00 AM • 10:00 AM	Plenary 3: Innate Immunity
	a. TLR Overview: Genes/Structure/Function - *TBD*
	b. TLR Polymorphisms and Defective Signalling - *Bruce Beutler*
	c. TLR in Disease/Autoimmunity NOD2 - *Judy Cho*
	Exhibit Hall Open and Posters
10:30 AM • 12:30 PM	Plenary 4: Signalling
	a. Immunological Synapse - *Michael Dustin*
	b. TcR Signalling - *TBD*
	c. Cytokine Signalling - *TBD*
	d. Targets of Immunosuppression - *Leslie Miller*
12:30 PM • 2:00 PM	ITN Lunch Symposium 1
2:00 PM • 4:00 PM	Symposium 2: Other Gene Families
	a. MIC Genes - *Seiamak Bahram*
	b. Cytokine Genes - *Ian Hutchinson*
	c. Polycystic Kidney Disease - *Peter Harris*
	d. Mutation Associated with the Hypercoagulable State - *Alexander Duncan*
	Workshop #3 - Case Studies - *Diane Pidwell*
4:30 PM • 6:00 PM	Abstracts #3
	Abstracts #4
	Workshop #4 - Cytokine Polymorphism, Expression & Detection - Elispot, PCR, Flow, Miltenyi - *TBD*
	Workshop #5 - ELISA - *Pat Adams*
6:00 PM • 7:30 PM	Wine and Exhibitor/Poster Session #2

Tuesday, October 22, 2002

7:00 AM • 5:00 PM	Registration
8:00 AM • 10:00 AM	Plenary 5: New Approaches to Autoimmunity
	a. T Regulatory Cells - *TBD*
	b. T Regulatory Cells in Autoimmune Disease (MS) - *David Hafler*
	c. Rheumatoid Arthritis-NOD - *Christophe Benoist*
	d. C' gene Polymorphism in Susceptibility to SLE - *Joseph Ahearn*
	Exhibit Hall Open and Posters
10:30 AM • 12:30 PM	Joint Symposium 3: (ASHI & Clinical Cytometry Society) Flow Cytometry Applications for Stem Cells
	a. Hematopoietic Stem Cells - *TBD*
	b. Non-hematopoietic Stem Cells - *TBD*
	c. Gene Therapy - *TBD*
	Workshop #6 - Advanced DNA - *Amy Hahn*
12:30 PM • 2:30 PM	President's Address
	Rose Payne Award and Lecture
2:30 PM • 4:00 PM	Symposium 4: International Histocompatibility Congress Update
	Workshop #7 - Internet Informatics Resources - *Wolfgang Helmberg*
	Abstracts #5
	Abstracts #6
4:30 PM • 6:00 PM	Workshop #8 - Post Transplant Monitoring and Engraftment - *Adriana Zeevi & David Senitzer*
	Workshop #9 - Tetramer Analysis of Autoreactive T cells in IDDM - *Bill Kwok*
	Abstracts #7
	Abstracts #8
6:15 PM • 7:45 PM	ASHI Annual Business Meeting

Wednesday, October 23, 2002

7:00 AM • 4:30 PM	Registration
7:00 AM • 8:30 AM	Laboratory Directors' Forum
	Technologists' Forum
8:30 AM • 10:30 AM	Plenary 6: B Cells
	a. B Cell Ontogeny - *Richard Hardy*
	b. Signalling Through the BcR and MHC Class II - *John Cambier*
	c. FcR Gene Family and Regulation of B Cells - *Jeffrey Ravetch*
11:00 AM • 1:00 PM	Plenary 7: B Cells Part II
	a. FcR Receptor Polymorphism
	b. FcR Polymorphism & Postengagement Signalling - *Bob Kimberly*
	c. B Cell Tolerance & Immunosuppresion - *TBD*
2:30 PM • 4:30 PM	Plenary 8 - Pathology & Immune Modulation of Antibodies
	a. C4d Deposition - *Robert Colvin*
	b. Signalling Through MHC - Class I - *Elaine Reed*
	c. IvIG - *Stan Jordan*
	d. Plasmapheresis & IvIG - *Lloyd Ratner*
5:00 PM • 6:00 PM	President's Reception
6:00 PM • 11:00 PM	Annual Banquet and Show on the General Jackson Riverboat

Times, speakers and final program subject to change

ASHI
American Society for Histocompatibility & Immunogenetics

3. Boyington, J. C., Motyka, S. A., Schuck, P., Brooks, A. G., and Sun, P. D. (2000). Crystal structure of an NK cell immunoglobulin-like receptor in complex with its class I MHC ligand, Nature 405, 537-43.

4. Braud, V. M., Allan, D. S., O'Callaghan, C. A., Soderstrom, K., D'Andrea, A., Ogg, G. S., Lazetic, S., Young, N. T., Bell, J. I., Phillips, J. H., et al. (1998). HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C, Nature 391, 795-9.

5. Colonna, M., Borsellino, G., Falco, M., Ferrara, G. B., and Strominger, J. L. (1993). HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells, Proc Natl Acad Sci U S A 90, 12000-4.

6. Dohring, C., Samaridis, J., and Colonna, M. (1996a). Alternatively spliced forms of human killer inhibitory receptors, Immunogenetics 44, 227-30.

7. Dohring, C., Scheidegger, D., Samaridis, J., Cella, M., and Colonna, M. (1996b). A human killer inhibitory receptor specific for HLA-A1,2, J Immunol 156, 3098-101.

8. Fan, Q. R., Long, E. O., and Wiley, D. C. (2001). Crystal structure of the human natural killer cell inhibitory receptor KIR2DL1-HLA-Cw4 complex, Nat Immunol 2, 452-60.

9. Gardiner, C. M., Guethlein, L. A., Shilling, H. G., Pando, M., Carr, W. H., Rajalingam, R., Vilches, C., and Parham, P. (2001). Different NK cell surface phenotypes defined by the DX9 antibody are due to KIR3DL1 gene polymorphism, J Immunol 166, 2992-3001.

10. Guidotti, L. G., and Chisari, F. V. (1996). To kill or to cure: options in host defense against viral infection, Curr Opin Immunol 8, 478-83.

11. Gumperz, J. E., Litwin, V., Phillips, J. H., Lanier, L. L., and Parham, P. (1995). The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor, J Exp Med 181, 1133-44.

12. Hughes, A. L., and Nei, M. (1988). Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection, Nature 335, 167-70.

13. Khakoo, S. I., Rajalingam, R., Shum, B. P., Weidenbach, K., Flodin, L., Muir, D. G., Canavez, F., Cooper, S. L., Valiante, N. M., Lanier, L. L., and Parham, P. (2000). Rapid evolution of NK cell receptor systems demonstrated by comparison of chimpanzees and humans, Immunity 12, 687-98.

14. Lanier, L. L. (1998). NK cell receptors, Annu Rev Immunol 16, 359-93.

15. Lanier, L. L., Corliss, B. C., Wu, J., Leong, C., and Phillips, J. H. (1998). Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells, Nature 391, 703-7.

16. Li, P., Morris, D. L., Willcox, B. E., Steinle, A., Spies, T., and Strong, R. K. (2001). Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA, Nat Immunol 2, 443-51.

17. Litwin, V., Gumperz, J., Parham, P., Phillips, J. H., and Lanier, L. L. (1994). NKB1: a natural killer cell receptor involved in the recognition of polymorphic HLA-B molecules, J Exp Med 180, 537-43.

18. Ljunggren, H. G., and Karre, K. (1990). In search of the 'missing self': MHC molecules and NK cell recognition, Immunol Today 11, 237-44.

19. Long, E. O., Colonna, M., and Lanier, L. L. (1996). Inhibitory MHC class I receptors on NK and T cells: a standard nomenclature, Immunol Today 17, 100.

20. Marsh, S. G., Bodmer, J. G., Albert, E. D., Bodmer, W. F., Bontrop, R. E., Dupont, B., Erlich, H. A., Hansen, J. A., Mach, B., Mayr, W. R., et al. (2001). Nomenclature for factors of the hla system, 2000, Hum Immunol 62, 419-68.

21. Moretta, A., Sivori, S., Vitale, M., Pende, D., Morelli, L., Augugliaro, R., Bottino, C., and Moretta, L. (1995). Existence of both inhibitory (p58) and activatory (p50) receptors for HLA-C molecules in human natural killer cells, J Exp Med 182, 875-84.

22. Parham, P., and Ohta, T. (1996). Population biology of antigen presentation by MHC class I molecules, Science 272, 67-74.

23. Pende, D., Biassoni, R., Cantoni, C., Verdiani, S., Falco, M., di Donato, C., Accame, L., Bottino, C., Moretta, A., and Moretta, L. (1996). The natural killer cell receptor specific for HLA-A allotypes: a novel member of the p58/p70 family of inhibitory receptors that is characterized by three immunoglobulin-like domains and is expressed as a 140-kD disulphide-linked dimer, J Exp Med 184, 505-18.

24. Ponte, M., Cantoni, C., Biassoni, R., Tradori-Cappai, A., Bentivoglio, G., Vitale, C., Bertone, S., Moretta, A., Moretta, L., and Mingari, M. C. (1999). Inhibitory receptors sensing HLA-G1 molecules in pregnancy: decidua- associated natural killer cells express LIR-1 and CD94/NKG2A and acquire p49, an HLA-G1-specific receptor, Proc Natl Acad Sci U S A 96, 5674-9.

25. Radaev, S., Rostro, B., Brooks, A. G., Colonna, M., and Sun, P. D. (2001). Conformational plasticity revealed by the cocrystal structure of NKG2D and its class I MHC-like ligand ULBP3, Immunity 15, 1039-49.

26. Rajagopalan, S., and Long, E. O. (1999). A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells, J Exp Med 189, 1093-100.

27. Rajalingam, R., Gardiner, C. M., Canavez, F., Vilches, C., and Parham, P. (2001a). Identification of seventeen novel KIR variants: fourteen of them from two non-Caucasian donors, Tissue Antigens 57, 22-31.

28. Rajalingam, R., Hong, M., Adams, E. J., Shum, B. P., Guethlein, L. A., and Parham, P. (2001b). Short KIR haplotypes in pygmy chimpanzee (Bonobo) resemble the conserved framework of diverse human KIR haplotypes, J Exp Med 193, 135-46.

29. Rajalingam, R., Krausa, P., Shilling, H. G., Stein, J. B., Balamurugan, A., McGinnis, M. D., Cheng, N. W., Mehra, N. K., and Parham, P. (2002). Distinctive KIR and HLA diversity in a panel of north Indian Hindus, Immunogenetics 53, 1009-19.

30. Shilling, H. G., Guethlein, L. A., Cheng, N. W., Gardiner, C. M., Rodriguez, R., Tyan, D., and Parham, P. (2002). Allelic polymorphism synergizes with variable gene content to individualize human KIR genotype, J Immunol 168, 2307-15.

31. Steffens, U., Vyas, Y., Dupont, B., and Selvakumar, A. (1998). Nucleotide and amino acid sequence alignment for human killer cell inhibitory receptors (KIR), 1998, Tissue Antigens 51, 398-413.

32. Suto, Y., Ishikawa, Y., Kasahara, M., Kasai, F., Yabe, T., Akaza, T., and Juji, T. (1998). Gene arrangement of the killer cell inhibitory receptor family on human chromosome 19q13.4 detected by fiber-FISH, Immunogenetics 48, 235-41.

33. The MHC sequencing consortium (1999). Complete sequence and gene map of a human major histocompatibility complex., Nature 401, 921-3.

34. Uhrberg, M., Valiante, N. M., Shum, B. P., Shilling, H. G., Lienert-Weidenbach, K., Corliss, B., Tyan, D., Lanier, L. L., and Parham, P. (1997). Human diversity in killer cell inhibitory receptor genes, Immunity 7, 753-63.

35. Valiante, N. M., Uhrberg, M., Shilling, H. G., Lienert-Weidenbach, K., Arnett, K. L., D'Andrea, A., Phillips, J. H., Lanier, L. L., and Parham, P. (1997). Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors, Immunity 7, 739-51.

36. Vilches, C., Rajalingam, R., Uhrberg, M., Gardiner, C. M., Young, N. T., and Parham, P. (2000). KIR2DL5, a novel killer-cell receptor with a D0-D2 configuration of Ig-like domains., J Immunol 164, 5797-804.

37. Wagtmann, N., Biassoni, R., Cantoni, C., Verdiani, S., Malnati, M. S., Vitale, M., Bottino, C., Moretta, L., Moretta, A., and Long, E. O. (1995). Molecular clones of the p58 NK cell receptor reveal immunoglobulin- related molecules with diversity in both the extra- and intracellular domains, Immunity 2, 439-49.

38. Wilson, M. J., Torkar, M., Haude, A., Milne, S., Jones, T., Sheer, D., Beck, S., and Trowsdale, J. (2000). Plasticity in the organization and sequences of human KIR/ILT gene families, Proc Natl Acad Sci U S A 97, 4778-83.

39. Winter, C. C., Gumperz, J. E., Parham, P., Long, E. O., and Wagtmann, N. (1998). Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition, J Immunol 161, 571-7.

40. Yabe, T., McSherry, C., Bach, F. H., Fisch, P., Schall, R. P., Sondel, P. M., and Houchins, J. P. (1993). A multigene family on human chromosome 12 encodes natural killer-cell lectins, Immunogenetics 37, 455-60.

41. Zinkernagel, R. M., and Doherty, P. C. (1974). Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system, Nature 248, 701-2.

J. Marilyn MacQueen

J. Marilyn MacQueen, an internationally recognized technical authority in the field of histocompatibility (tissue matching), died Thursday at her home in Hillsborough, NC. She was the daughter of the late Julia Smith and James E. MacQueen of the Carolina section of Dillon County, SC.

Marilyn was born in Bennettsville, SC, August 7, 1939. A graduate of Dillon High School, Dillon, SC, she earned her BA in Biology in 1961 at Queens College, Charlotte, NC. Marilyn was given an honorary Doctor of Humane Letters degree by Queens College in 1987 for her extraordinary contributions to her profession and her enthusiastic involvement in and support of her college since graduation. Marilyn was an undergraduate laboratory instructor at Queens College (1959-61). She taught science and biology in the Ocala, Florida, school system (1961-64). Returning to North Carolina in 1964, she was a research associate in the Duke University Department of Immunology and later in the fledgling area of Histocompatability and Immunogentics. In 1969 she was appointed supervisor of the Durham Veterans Administration Medical Center (DVAMC) Transplantation Laboratory and held that position until her retirement in 1997. As laboratory supervisor, she and her staff were responsible for tissue typing all transplantation patients at the DVAMC, Duke University Medical Center and UNC Hospitals.

Recognized internationally in the field of histocompatibility and organ transplantation, she participated in four of the earliest International Histocompatibility Workshops to expand the network of academic expertise to Europe, Australia, New Zealand and beyond. She was instrumental in the early development of all areas in the field of Histocompatibility during the era when Duke University was the focal point in the origination of this vast field. She was consistently a pioneer, teacher, innovator, creative force and consultant. She was the person who "made things happen." She led field investigations for the NIH, SEOPF and Duke department of Histocompatibility and Immunogenetics in such diverse areas as the Artic region of Finland, the outback of Australia and the Cook Islands.

Marilyn was a founding member and active participant in numerous professional organizations, including the Southeastern Organ Procurement Foundation (SFOPF), the American Association for Clinical Histocompatibility Testing (AACHT), the American Society of Histocompatibility and Immunogenetics (ASHI), Carolina Donor Services (CDS- formerly the Carolina Organ Procurement Agency), the American Association of Blood Banks (AABB) and the United Network Organ Sharing (UNOS) which is the current organization for the equitable placement of transplant organs throughout the U.S.

Marilyn received a multitude of professional honors and awards including undergraduate recipient of Who's Who in American Colleges and Universities (1961), the National Science Foundation Study Fellowship (1963), the Upjohn/ Southeastern Organ Procurement Foundation (SEOPF) Award (1977) and SEOPF's Meritorious Achievement Award (1981 and 1987). She was the first recipient of Outstanding Technologist Award (1987) given by ASHI, an agency that she helped develop. Today, ASHI is the internationally recognized organization responsible for licensing, teaching and assuring high quality in all transplant programs. In 1988, Marilyn received the SEOPF Special Achievement Award for her exceptional work in development of a network for support and interaction with tissue typing labs in the Southeast area.

Marilyn co-authored many articles published in national and international medical journals and texts and edited two editions of the SEOPF Tissue Typing Procedure Manual that are still invaluable teaching tools for new members of this field. Since her retirement, she was actively involved in the Orange Congregations in Mission Meals on Wheels program.

Surviving are her life partner Barbara O. Burgess of Hillsborough, NC; one step-brother Rene DeLind, of Moreno Valley, CA; first cousins Alexander McRae MacDonald of Clio, SC, Donald Frank MacDonald of Edinburgh, Scotland, and Robert McLaurin MacDonald of Lakeland, FL; and numerous nieces, nephews and cousins. Marilyn also leaves an immense community of friends and acquaintances who have been forever changed by having had the experience of knowing this extraordinary and unique life. Her uniqueness, as often described by her friends, is " the mold was broken after Marilyn was created."

In lieu of flowers, donations may be made to Orange Congregations in Mission, 300 Millstone Drive, Hillsborough, NC 27278 (Designate Meals on Wheels); Carolina Presbyterian Church, 2971 Calhoun Road, Clio, SC 29525; or Queens College, (attention: Adelaide Anderson Davis), 1900 Selwyn Avenue, Charlotte, NC 28208.

Tech Tips

Effect Of Thymoglobulin® On Immune Monitoring and Crossmatch Interpretation

J. McKie[1], W. Shumway[1], D. Rinde-Hoffman[2], and W. LeFor1 - LifeLink Transplantation Immunology Laboratory[1], LifeLink Institute[2], Tampa, FL

Thymoglobulin® (rabbit anti-human thymocyte globulin or ATG) is commonly used in post-transplant patients as part of their immunosuppressive protocol. The presence of these antibodies in patient sera produces false positive reactions in crossmatches making post-transplant immune monitoring difficult. ATG binds to T-cells and fixes complement, thereby giving false positive reactions in the cytotoxic crossmatch (CDC-XM). In the flow crossmatch (FC-XM), many widely used secondary antibodies (we use goat anti-human IgG) will bind to the ATG antibody bound to donor cells, again resulting in a false positive result. We reasoned that neutralization or removal of residual ATG would eliminate such false positive crossmatch results.

Our lab has developed a protocol by which ATG can be removed from test serum by adsorption with superparamagnetic polystyrene beads coated in donkey anti-rabbit IgG. The effectiveness of these beads was tested with ATG diluted to a concentration representative of the highest levels expected in dosed patients, as well as with this diluted ATG mixed with human serum that has known HLA antibody. The results showed that the beads removed all reactivity due to ATG but that they had no effect on reactivity due to anti-HLA. Shown below is one example of our use of this protocol.

Example:

A 21 year old transfused but non-sensitized (CDC-PRA = 0 percent, FC-PRA Class I&II = 0 percent) Caucasian female received a cadaver heart transplant. Per protocol, crossmatching was not performed for the non-sensitized patient. The HLA mismatches were A1, A2, B62, DR1, DR4, DRw53, and DQ7.

Six days post-transplant the heart was failing and she was re-activated at critical status for re-grafting. Serum screened for antibody at this time showed FC-PRA Class I = 90 percent Class II = 38 percent. Class I specificity showed strongest reactions for B62-35-75-5, with weaker reactions following the pattern for A1-3-9-11 and A2. Class II specificity showed a clear DRw53 and DQ2.

Ten days post-transplant a heart became available and crossmatches with surrogate first donor (no actual first donor cells available) and intended second donor were performed. The patient was receiving Thymoglobulin® in 50mg doses as part of her immunosuppressive therapy, therefore her post 1st graft serum was adsorbed according to our protocol. The crossmatch results were as follows:

- Surrogate 1st donor (representing mm Ag A2, B62, DR4 and DRw53)
  - FC-XM T&B very strongly positive with untreated AND adsorbed serum
- Intended 2nd donor (mm for A31, B38 and DR103 → antigens NOT related to first donor and NOT included in the antibody specificities identified post 1st graft)
  - CDC-XM negative (ATG concentrations well below detectable cutoff)
  - FC-XM T&B strong positive with untreated serum, T=/+ and B+ (reduced to approx. 25 percent of untreated serum vs. T cells) with adsorbed serum

The sum total of information to this point indicated to us that the patient has a very high titre of antibody to the mismatched Class I and Class II antigens from donor #1 and may possibly have very low levels of Class I and a moderate amount of Class II antibody to the intended donor #2. Taking into account the patient was very critical and since increased B cell fluorescence in the flow crossmatch is often NOT due to HLA antibody, the surgeons would likely have considered these results acceptable for transplant. However, in our lab we do not just look at increased fluorescence. We evaluate the nature of the binding that causes the fluorescence by using methods presented at previous ASHI meetings. These include adsorbing sera with donor cells and/or platelets, as well as diluting or increasing the amount of sera added in the crossmatch. All of those manipulations have predictable effects on true Ab/Ag interactions. If these effects are not observed, we can then conclude that the observed binding is not due to a specific Ab/Ag interaction and therefore is not due to HLA. The observed T & B binding against the intended 2nd donor did not behave like a specific Ab/Ag interaction and therefore the crossmatch was called negative.

The patient was discharged from the hospital three and a half weeks after the second transplant and is currently doing well 8 months post-transplant.

Summary of the Northeast Regional Workshop, Baltimore, MD April 10-12, 2002

John Hart, MBA, CHS and Lori Osowski, MS, CHS

The Northeast Regional Workshop took place April 11-12, 2002 in Baltimore, MD at the stunning Marriott Waterfront Hotel. The American Red Cross National Histocompatibility Laboratory and the Johns Hopkins University Immunogenetics Laboratory hosted the workshop. There were 136 attendees. The Accreditation Program sponsored an Inspector Training Workshop for current ASHI inspectors preceding the start of the workshop on April 10th. Kathy Muto, chair of Inspector Training, presented a thorough review of current policies and issues that affect the performance of inspections. Andrea Zachary detailed troublesome areas in the inspection process and provided pointers on specific areas including DNA, ELISA and Flow cytometry inspections. John Hart provided an update on the continued drive to develop an electronic format for the inspection checklist and process. There were 15 attendants. Later that evening, the actual workshop began with a welcome reception, which provided an opportunity for attendees to meet their colleagues.

On Thursday, April 11, the workshop opened with a session called "Recent Advances in Immunogenetics and Immunobiology." The session included an exciting state of the art talk by Peter Creswell, Ph.D. regarding "Recent Developments in Antigen Processing" and a very lively and spontaneous discussion about an alternative theory to how the immune system functions, termed, "the Danger Hypothesis" so fondly named by speaker, Polly Matzinger, Ph.D. The morning concluded with Session II, Clinical Applications in Immunogenetics, which included a very informative talk about "HLA Polymorphism and Infectious Diseases" by Xiaojiang Gao, Ph.D. and "Emerging Issues in Blood and Marrow Transplantation" by Neil Flomenberg, M.D.

The afternoon session, "HLA Antibodies in Transplantation," was very exciting and gave way to very interesting discussion by day's end. Donna Lucas, CHS gave a nice overview of the Impact of Antibodies on Organ Transplants. Next, Dessislava Kopchaliiska, Ph. D. and Lori Osowski, CHS, gave updates of the Johns Hopkins and University of Maryland/ARC Renal Desensitization Protocols. This was followed by a "round robin" group of speakers, Meg Gobeli, CHS, Julie Graziani, CHT, Beverly Muth, CHT and Jeffrey Sholander, CHS. They gave a wonderful collective summary of the "Relevance and Application of Antibody Tests - Characteristics of High Risk Antibodies". Finally, the afternoon was wrapped up with an expert panel (Shirley M. Polly, MD, Nancy Reinsmoen, Ph.D., Gary A. Teresi, CHS) commenting on interesting case studies presented by meeting attendees, Lynne L. Klingman, CHS, Sandy Rosen- Bronson, Ph.D., Jeannie Roule, CHS, Donna Fitzpatrick, CHS and Barbara Burgess, CHS.

After a jam-packed day of science, the meeting was concluded and everyone took a break by enjoying a stroll along the Inner Harbor, dinner at the Camden Yards Bull Pen followed by a rousing Baltimore Orioles baseball game vs. the Tampa Bay Devil Rays. The Orioles cooperated by winning!!!! Baltimore 15 - Tampa Bay 6. It was an exciting game that had the fans guessing until the 6th inning, when the Orioles wowed everyone with 12 runs!

On Friday, April 12, the sessions began with Current Issues in Immunogenetics. Mary S. Leffell, Ph.D., ABHI gave a comprehensive, excellent review of "Natural Killer/Leukocyte Receptor Complexes", followed by the interesting and informative topic of "Access Issues in Renal Transplantation" by Milagros Samaniego-Picota, M.D. This was followed by "CLIA Update - Centers for Medicare & Medicaid Services" by Cecilia Hinkel, MT (ASCP) attending from CMS.

For lunch, the attendants enjoyed the wonderful and touching address by a keynote speaker, the Acting Deputy Surgeon General of the United States, Kenneth P. Moritsugu, M.D., MPH. Dr. Moritsugu shared Perspectives from the Office of the Surgeon General that included his personal experiences with the transplant process and a tribute to all laboratory professionals who work behind the scenes to make organ transplantation a reality. His commentary was very uplifting for everyone.

The meeting ended with a bang. The final session, Generation and Application of Molecular Data, began with a very comprehensive discussion of HLA DNA Platforms by Debra L. Kukuruga, Ph.D. This was followed by an interesting presentation about Micro-Array Technology by Kevin Becker, Ph.D. and the finale speaker Marcelo Fernandez-Vina, Ph.D., with a detailed discussion of Donor Search Strategies for Allogeneic Marrow Transplantation. Throughout the meeting, posters presented at the last annual ASHI meeting were available for review.

The corporate sponsors had an opportunity to present their "Innovative Technology" to the attendants in three sessions throughout the meeting. We thank them for their generous support of the workshop.

In summary, the meeting was well received from many standpoints. The level of scientific and technical content, the quality of speakers, the location and the structure of the program combined to create a successful workshop. We appreciated all the help and support from many individuals who participated both on the program and behind the scenes. Special thanks to the staff members from Association Headquarters, Len Morrissey, Karen Feder and Emily Rosenberg.

2002 ASHI Officers and Council Members

PRESIDENT
Robert A. Bray, PhD, D(ABHI) (2003)
Emory University Hospital
Dept of Pathology, Rm F-149
1364 Clifton NE
Atlanta, GA 30322
(404) 712-7317
Fax: (404) 727-1579
E-mail: rbray@emory.edu

PRESIDENT ELECT
Adriana Zeevi, PhD, D(ABHI) (2004)
University of Pittsburgh Medical Center
Biomedical Sci Tower Rm W1552
Lothrop & Terr Sts
Pittsburgh, PA 15261
(412) 624-1073
Fax: (412) 624-6666
E-mail: zeevi+@pitt.edu

PAST PRESIDENT
Dolly B. Tyan, PhD, D(ABHI) (2002)
Cedars-Sinai Medical Center
Medical Genetics, SSB-378
8700 Beverly Blvd
Los Angeles, CA 90048
(310) 423-4979
Fax: (310) 423-0391
E-mail: tyand@cshs.org

VICE PRESIDENT
Sandra Rosen-Bronson, PhD, D(ABHI) (2002)
Georgetown University Hospital
3900 Reservoir Road, NW, Rm LE811-Preclinical Science Building
Washington, DC 20016
(202) 687-8924
Fax: (202) 687-1244
E-mail: bronson@gunet.georgetown.edu

SECRETARY
William W. Ward, PhD, D(ABHI) (2004)
MedStar Research Institute
Transplant & Immunogenetics Lab
108 Irving St, NW
Washington, DC 20010
(202) 877-6549
Fax: (202) 877-6136
E-mail: William.w.ward@medstar.net

TREASURER
Donna L. Phelan, BA, CHS, MT(HEW) (2003)
Barnes-Jewish Hospital Labs
One Barnes Plaza
St Louis, MO 63110
(314) 362-6527
Fax: (314) 362-4647
E-mail: dlphelan@aol.com

COUNCILOR AT LARGE
Amy B. Hahn, PhD, D(ABHI) (2003)
Albany Medical College Trans Immunology
47 New Scotland Ave
Rm ME524 Mail Code 62
Albany, NY 12208
(518) 262-5574
Fax: (518) 262-6274
E-mail: hahna@mail.amc.edu

COUNCILORS
Ann B. Begovich, PhD (2004)
Celera Diagnostics
1401 Harbor Bay Parkway
Alameda, CA 94502
(510) 749-4243
Fax: (510) 749-5200
E-mail: Ann.begovich@celeradiagnostics.com

Frans Claas, PhD (2004)
Immunohematology & Blood Transf
Bldg 1-E3-Q
LUMC PO Box 9600
Leiden, 2300RC
Netherlands
011-31-71-526-3800
Fax: 011-31-71-521-6751
E-mail: fhjclaas@lumc.nl

Marcelo Fernandez-Vina, PhD, D(ABHI) (2002)
C.W. Bill Young DoD Marrow Program
Naval Medical Research Center
Georgetown University
5516 Nicholson Lane, Building A
Kensington, MD 20895
(301) 998-8900
Fax: (301) 998-8946
E-mail: mfervina@erols.com

Joan E. Holcomb, MS, CHS (2002)
Emory University Hospital
HLA Lab Room C184
1364 Clifton Rd NE
Atlanta, GA 30322
(404) 712-7365
Fax: (404) 712-4717
E-mail: Joan_bray_holcomb@emory.org

Cathi L. Murphey, MT(ASCP), CHS, DLM (2003)
Southwest Immunodiagnostics
8122 Datapoint Dr
Ste 912
San Antonio, TX 78229
(210) 614-3703
Fax: (210) 614-3707
E-mail: murphy@onr.com

Paula J. Romano, PhD (2002)
Milton S. Hershey Medical Center
Dept of Pathology, H140
PO Box 850
Hershey, PA 17033
(717) 531-5117
Fax: (717) 531-5409
E-mail: promano@psu.edu

Susan Saidman, PhD, D(ABHI) (2002)
Massachusetts General Hospital
Histocompatibility Lab
55 Fruit St, Rm WHT544
Boston, MA 02114
(617) 724-3767
Fax: (617) 724-3331
E-mail: ssaidman@partners.org

Victoria Turner, PhD, D(ABHI) (2004)
St Jude Hospital
HLA Lab C4035
332 N Lauderdale
Memphis, TN 38105-2794
(901) 495-3468
Fax: (901) 495-3100
E-mail: Vicky.turner@stjude.org

2001 - 2002 ASHI Committee Chairs

FINANCE AND AUDIT COMMITTEE
Howard Gebel, PhD, D(ABHI)
Emory University Hospital
1364 Clifton Road, NE
Room F-149
Atlanta, GA 30322
(404) 712-7308
Fax: (404) 727-1579
E-mail: hgebel@emory.edu

BYLAWS COMMITTEE
Lori Osowski, MS, CHS
Supervisor
American Red Cross National Histocompatibility Lab
22 S. Greene St., Box 173
Baltimore, MD 21201-1595
(410) 328-2973
Fax: (410) 328-2967
E-mail: oswoskil@usa.redcross.org

MEMBERSHIP, MARKETING AND PUBLIC RELATIONS COMMITTEE
Nancy Reinsmoen, PhD, D(ABHI)
Director, Transplant Immunology Lab
Duke University Medical Center
Pathology Dept.-Box 3712DUMC
Research Park Bldg. III Research Dr.
Durham, NC 27710
(919) 684-3089
Fax: (919) 684-9089
E-mail: reins001@mc.duke.edu

TECHNOLOGISTS' AFFAIRS COMMITTEE
Nancy Higgins, MT, CHS
Methodist Hospital
1701 N. Senate Blvd.
Transplant Immunology WH116
Indianapolis, IN 46202
(317) 962-6194
Fax: (317) 962-6195
E-mail: nhiggins@clarian.com

DIRECTORS' AFFAIRS COMMITTEE
Sandra L. Nehlsen-Cannarella, PhD
Loma Linda University Medical Center
11234 Anderson St., Room 2578
Loma Linda, CA 92354-2870
(909) 558-4144
Fax: (909) 558-0144
E-mail: sncannarella@ahs.llumc.edu

PUBLICATIONS COMMITTEE
Robert E. Lewis, PhD
Co-Director, Immuno Path/ Transplant
University of Mississippi Medical Center
2500 N. State St.
Department of Pathology
Jackson, MS 39216-4505
(601) 984-1562
Fax: (601) 984-1835
E-mail: rlewis@pathology.umsmed.edu

WEB COMMITTEE
William W. Ward, PhD, D(ABHI)
MedStar Research Institute
Transplant & Immunogenetics Lab
108 Irving St., NW
Washington, DC 20010
(202) 877-6549
Fax: (202) 877-6136
E-mail: william.w.ward@medstar.net

SCIENTIFIC AFFAIRS COMMITTEE
Peter Nickerson, PhD
Canadian Blood Services
Winnipeg Centre
777 William Avenue
R3E 3R4 Canada
(204) 789-1130
Fax: (204) 783-6780
E-mail: peter.nickerson@bloodservices.ca

EDUCATION COMMITTEE
Cathi Murphey, MT(ASCP), CHS, DLM
Southwest Immunodiagnostics
8122 Datapoint Dr., Ste. 912
San Antonio, TX 78229
(210) 614-3703
Fax: (210) 614-3707
E-mail: murphy@onr.com

INTERNATIONAL AFFAIRS COMMITTEE
Chaim Brautbar
Hadassah Medical Center
P.O. Box 12000
Tissue Typing Unit
Jerusalem, 91120, Israel
011-972-2-6777801
Fax: 011-972-2-6422403
E-mail: brautbar@hadassah.org.il

QUALITY ASSURANCE/ STANDARDS COMMITTEE
Robert O. Endres, PhD, D(ABHI)
Blood Systems Labs
HLA Lab
2424 W Erie Dr.
Tempe, AZ 85282
(480) 675-7092
Fax: (480) 675-7025
E-mail: rendres@bloodsystems.org

PROFICIENCY TESTING COMMITTEE
Thomas M. Williams, PhD
University of New Mexico Health Sciences Center
915 Camino de Salud, NE
Rm. 337-BMSB, Department of Pathology
Albuquerque, NM 87131-5301
(505) 272-8059
Fax: (505) 272-9038
E-mail: twilliams@salud.unm.edu

ACCREDITATION REVIEW BOARD
Geoffrey Land, HCLD, PhD
Methodist Medical Center
1441 N. Beckley Ave.
Dallas, TX 75203
(214) 947-3584
Fax: (214) 947-3598
E-mail: galand@flash.net

LABORATORY DIRECTORS' TRAINING REVIEW SUBCOMMITTEE
Sandra Helman, PhD, D(ABHI)
Medical College of GA Hospital & Clinics
1120 Fifteenth St.
BAS 1641
Augusta, GA 30912-4091
(706) 721-3311
Fax: (706) 721-2709
E-mail: shelman@mail.mcg.edu

AWARDS COMMITTEE
Brian Duffy, MA, CHS
Barnes Jewish Hospital
HLA Lab, Mail Slot 90-23-335
One Barnes Plaza
St. Louis, MO 63110
(314) 747-0435
Fax: (314) 362-4647
E-mail: bdduff@aol.com

NATIONAL AFFAIRS/ PUBLIC POLICY COMMITTEE
Louise M. Jacobbi
Saturn Management Services
Legacy Donor Foundation
208 Glenwood Dr.
Metairie, LA 70005
(504) 835-2767
Fax: (504) 834-2069
E-mail: ljacobbi@aol.com

JUDICIARY COMMITTEE (AD HOC)
Beth Colombe, PhD, D(ABHI)
Associate Director
Thomas Jefferson University Hospital
Immunogenetics and Tissue Typing Lab
111 S. 11th St., Rm 8245 Gibbon Bldg.
Philadelphia, PA 19107
(215) 955-1136
Fax: (215) 923-8793
E-mail: beth.colombe@mail.tju.edu

NOMINATIONS COMMITTEE
Adriana Zeevi, PhD, D(ABHI)
University of Pittsburgh Medical Center
Biomedical Science Tower, Rm W 1552
Lothrop & Terr Sts.
Pittsburgh, PA 15261
(412) 624-1073
Fax: (412) 624-6666
E-mail: zeevi+@pitt.edu

2002 PROGRAM COMMITTEE
Dolly B. Tyan, PhD, D(ABHI)
Cedars-Sinai Medical Center
Medical Genetics, SSB-378
8700 Beverly Blvd.
Los Angeles, CA 90048
(310) 423-4979
Fax: (310) 423-0391
E-mail: tyand@cshs.org

New Members

NHL Quality Systems Support
22 S Greene St Box 173
Baltimore, MD 21201
(410) 328-2955
(410) 328-2967
hunterrj@usa.redcross.org

Jignesh Dalal, MD
Ped/Oncol CMSC-800
600 N Wolfe St
Baltimore, MD 21231
(410) 434-2001
(410) 955-0028
jdalal1@jhmi.edu

Pamela Doyle, BS, MT
Transplant Immunology Lab
80 Seymour StHartford, CT 06102-1537
(861) 545-3938
(860) 545-4356
pdoyle@hart.hosp.org

Carmen Edwards, BS,MT
715 Dryden St
Silver Spring, MD 20901
edwardsc@gunet.georgetown.edu

E Elango, M.Sc,PhD
Molecular Biology
Elamakara
Cochin Kerala, 682026
India
91-484-339080
91-484-340801
elango@aimshospital.org

Ann Green, MSC,BSc
Histocompatibility & Immunogen
Southmead rd
Bristol Avon B510 5RD,
England
0119-9912072
0119-9121514
ann.green@nbs.nhs.uk

Jean Hood, BS,ASCP
Tissue Typing
1616 Hayes StNashville, TN 37203
(615) 321-0221
(615) 321-4880
jhood66732@aol.com

Luiz Jobim, UFRGS,MD
Servico de Immunologia
Rua Ramiro Barcelos 2350
Porto Alegre RS 90035-003,
Brazil
55-51-33168020
55-51-33168001
drjobim@dnareference.com.br

Byung Kim, MD,PhD
Clinical Pathology
Dae Chun-Dong Jung-gu 4-12
Pusan,
South Korea
051-461-2279
051-247-6560
bchangkim@hotmail.net

Lora Offord, BS,CHT
Clin Immuno & Histocompat Lab
12635 E Montview Blvd Ste 224
Aurora, CO 80010
(303) 724-1300

Evan Ray, BA,SI,CHS,CHT
190 Ebbtide Dr
San Antonio, TX 78227
evan.ray@59mdw.whmc.af.mil

Lora Rice, BS,ASCP
Histocompatibility1616 Hayes St
Nashville, TN 37203
(615) 321-0212
(615) 321-4880

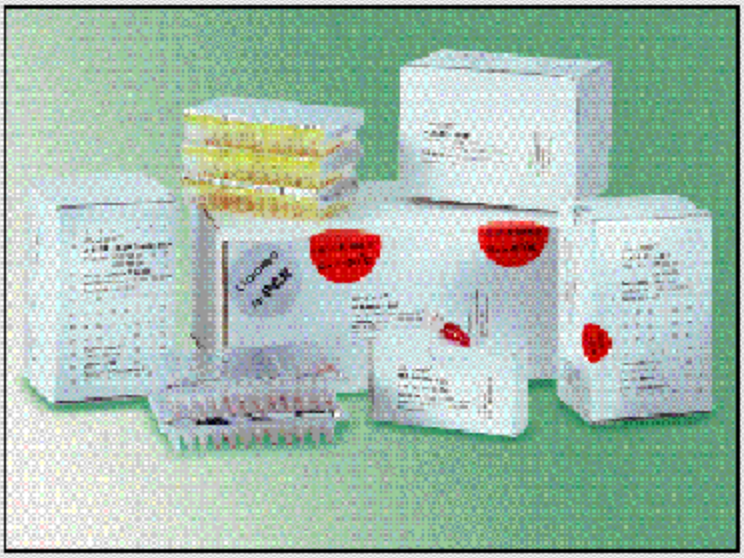
Thuydung Tu, BS,MT
HLA Lab Lab of Med rm LE8H
3900 Reservoir Rd NW
Washington, DC 20007
(202) 784-3550
(202) 784-3552
tut@gunet.georgetown.edu

Angie Turner, BS,ASCP
Tissue Typing
1616 Hayes St
Nashville, TN 37203
(615) 321-0212

Kristen Wolfe, BS,MS
Histocompat & Immun Eval Lab
333 Cedar St FMB 14
New Haven, CT 06510
(203) 785-5322
(203) 785-7617
kristen.wolfe@yale.edu

OLERUP SSP™ HLA Products

for Complete and Up-to-Date HLA Typing



Primer mixes are prepippeted into PCR tubes and PCR Master Mix includes all reagents except DNA and water.

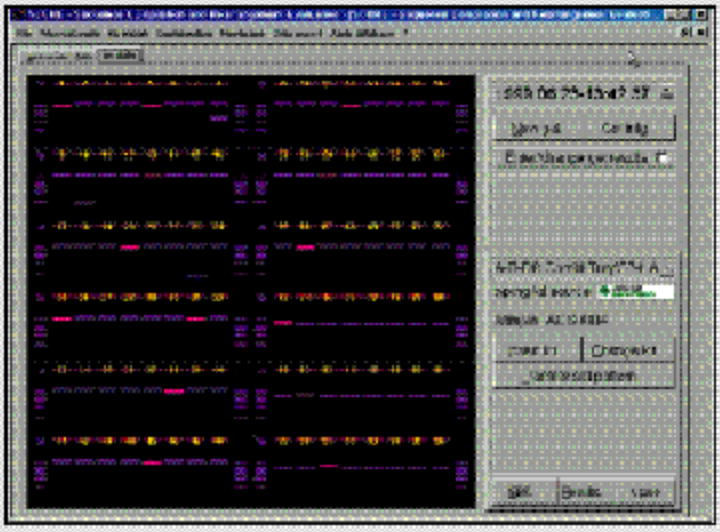
Products are available in two formats:
- Licensed: Includes TAQ Polymerase and PCR license.
- Unlicensed: without TAQ Polymerase and PCR license*.

* If purchasing without a PCR license it is the obligation of the user to obtain a PCR license from F. Hoffmann-LaRoche Ltd and Roche Molecular Systems, Inc.

The *OLERUP* SSP™ HLA Products are complete in every way:

- Complete product line for typing all classical HLA loci including A, B, C, DRA, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1 and DPB1.
- Complete set of reagents included in all licensed kits, just add DNA and water.
- Complete coverage of WHO recognized alleles in new lots as soon as new nomenclature updates are released, the most up-to-date SSP products available.
- Completely compatible with NMDP medium resolution typing requirements (Feb. 2001) for HLA-A, HLA-B and DRB.
- Complete line of accessory products for ease of use including a DNA size marker, negative control tubes, PCR wipe test, ethidium bromide dropper bottles and gel electrophoresis equipment.

Customized Heimberg - SCORE Virtual Sequencing Software available free-of-charge to all GenoVision *OLERUP* SSP™ customers.



- With the SCORE program, your typing is always up-to-date with the most current allele database because the analysis is based on the reactions with the target sequence motifs of the primers.
- Allele database frequently updated to all customers using SCORE.

GenoVision Inc.
[illegible] South Bolmar St., Suite R
West Chester, PA [illegible] USA
order@genovision.com
Order: +1 [illegible]
Fax: +1 [illegible]

GenoVision AS
Fryshuvien 40
N-[illegible] Oslo, Norway
order@genovision.no
Order: +47 [illegible]
Fax: +47 [illegible]

GenoVision Vertriebsgesellschaft
Lövergasse [illegible],
A-[illegible] Vienna, Austria
office@genovision.at
Order: +43 1 710 [illegible]
Fax: +43 1 710 [illegible]

GenoVision

www.genovision.com

ASHI Version

One System: Multiple Possibilities!

The new Lambda Array ... Multi-Analyte ...m™ (LABMAS) ... Luminex® ...ltiplex analysis technology to provide innovativ... ...utions for both ...rse SSO typing ... antibody ...tion. LABMAS™ features simple and fast procedu... ...nd even faste... analysis. Comp... ...to conv... ...al technologies, the time and effort to obtain you... ...ults is dramatic... ...educed.

For SSO Typin...

LABType™ SSO HLA Class I and Class II DNA typing system provides a complete solution for **A, B, DRB1, and DQB1* locus typing,** using a simple, straightforward procedure. This reliable test uses innovative and proven technology, which features high speed data acquisition and real-time multiplex analysis. LABType™ is ideal for both low and high throughput testing.

For Ant... ...Screening

LABScreen™ PRA, offers comprehensive Class I and Class II PRA screening and specificity assignment by flow analysis. Also available are **LABScreen™ Mixed,** our cost-effective, pre-screening test that can detect HLA Class I and Class II antibodies in a single tube protocol and **LABScreen™ Single Antigen** used for uncovering hidden specificities in high PRA sera.

For additional information on LABType™ SSO, LABScreen™ PRA or any of One Lambda's quality HLA products, contact your One Lambda representative or visit our web site.

One Lambda, Inc.

21001 Kittridge St., Canoga Park, CA 91303-2801, USA

TEL: 818/702-0042 800/822-8824 (except greater L.A. area)

FAX: 818/702-6904 800/992-2111 (U.S. and Canada only)

INTERNATIONAL: Contact your local distributor

www.onelambda.com

© Copyright 2002, One Lambda, Inc. All rights reserved.
* LABType™ DQB1 will be available fourth quarter, 2002.
™Lambda Array Beads Multi-Analyte System (LABMAS) is a trademark of One Lambda, Inc., LABType, LABScreen, LABScan 100 are trademarks of One Lambda, Inc.
All One Lambda software products are designed to assist personnel experienced in HLA analysis by suggesting screening or typing results. However, all suggested typings or screenings must be carefully reviewed by such qualified personnel to assure correctness. This software is meant as a laboratory aid, not as a source of definitive results.

LABMAS™ PRODUCTS

Sponsorship Opportunities

2002 ASHI Annual Meeting

October 19-23

Nashville, Tennessee

Are your products and services vital to the fields of histocompatibility and immunogentics?

If so, become a sponsor of the 2002 ASHI Annual Meeting being held at the Opryland Resort in Nashville Tennessee, October 19-23, and increase your visibility and exposure to the 1,000 clinical and research professionals in attendance.

Receive tangible benefits based on your contribution level including, but not limited to, promotional exposure in printed meeting materials. Levels offered, include:

- Platinum Level (Contribution of $15,000 +)
- Gold Level (Contribution of $8,000 +)
- Silver Level (Contribution of $5,000 +)
- Bronze Level (Contribution of $3500 +)
- Copper Level (Contribution of $1500 +)

Demonstrate your role and commitment to the science and advancement of histocompatibility and immunogenetics, by pledging a contribution today.

For a complete list of sponsor benefits and specific opportunities per level,

contact: Clare Rife, ASHI Exhibit Manager

17000 Commerce Parkway, Suite C,
Mt. Laurel, NJ 08054

Phone: 856-642-4425

Fax: 856-439-0525

E-mail: crife@ahint.com

Note: Deadline for sponsorship payment is August 14, 2002

All sponsor benefits are subject to availability based on time of reservation. Early reservations are strongly suggested in order for sponsor name to be included in pre-meeting and on-site promotion benefits.

The ASHI Proficiency Testing Program

- Administered by ASHI, the recognized leader in histocompatibility testing
- Designed to fully meet latest ASHI standards for laboratory accreditation
- Simplified submission and reporting of results; rapid turnaround of summary reports
- Modular configurations provide flexibility and cost savings; order only what's needed for your laboratory procedures
- Test samples provided by a laboratory with more than 20 years experience distributing proficiency specimens for histocompatibility testing
- Competitively priced

Five Testing Categories Offered

SEROLOGICAL TYPING FOR HLA-A, B, C, DR, DQ ± ABO GROUPING

- Subscribe to modules for class I and/or class II testing on same samples
- Optional ABO grouping module available

MOLECULAR TYPING FOR HLA-A, B, C, DR, DQ, DP

- Modules available for class I and/or class II testing on same specimens
- Perform low and/or high resolution level typing

HLA CROSSMATCHING / ANTIBODY SCREENING

- Perform characterization of class I antibody (with T cell crossmatches) and/or class II antibody (with B cell crossmatches)
- Order volumes to accommodate your test methods: lymphocytotoxicity testing (with or without AHG), flow cytometry, ELISA
- Report results obtained by multiple methods
- Includes clinical scenarios for increased educational opportunities

HLA-B27 DETECTION

- Identify presence or absence of HLA-B27 for disease association
- Use testing method(s) of your choice

ENGRAFTMENT MONITORING

- Detection of percent donor-recipient admixture in simulated post-transplant samples
- Report quantitative and/or qualitative results

THE AMERICAN SOCIETY FOR HISTOCOMPATIBILITY AND IMMUNOGENETICS

the recognized leader in
Clinical Histocompatibility Testing

announces the

ASHI PROFICIENCY TESTING PROGRAM

AVAILABLE IN JANUARY 2003

THE ENHANCED PROFICIENCY TESTING PROGRAM IS DESIGNED EXCLUSIVELY TO ADDRESS THE NEEDS OF THE CLINICAL HISTOCOMPATIBILITY LABORATORY

YES! I want more information on the ASHI Proficiency Testing Program for 2003. Please send me additional information and a subscription booklet for the following areas of testing:

____ Serological Typing for HLA-A,B,C,DR,DQ ____ Molecular Typing for HLA-A,B,C,DR,DQ,DP

____ HLA Crossmatching/Antibody Screening ____ HLA-B27 Detection ____ Engraftment Monitoring

Name_____Title_____

Laboratory_____

Address_____

_____

City_____State_____Zip_____

Telephone_____Fax_____E-mail_____

Return to ASHI Office at:

Fax: 856-439-0525

OR mail via return postage:

17000 Commerce Parkway
Suite C
Mt. Laurel, NJ 08054

For questions:

E-mail: ashi@ahint.com

The ASHI Web Site

www.ashi-hla.org

Ronald K. Charlton, PhD

This month I will continue our tour of the web site with a review of the "About ASHI," "Accreditation" and "ASHI Governance" areas. Before I start our tour, let me again mention the discussion groups ("Digital Discussions"). Postings in most of the discussion groups can be accessed and read without a login. For those few areas that are protected, you can establish your own profile (user account) with user name and password. Establishing your profile and registering for any public discussion group is instantaneous. For laboratory directors wishing to access the Director's Forum for the first time, the application is reviewed and approved within about 24 hours. For simplicity sake, you may want to establish a user profile using the same login as for the member-restricted areas of the web site, which is your last name and member number.

As indicated in the intro to the discussion site, there are advantages to establishing a profile for all of the discussion groups, particularly being able to receive notification of new posts via email. There are still occasional problems with logging on to the protected sites of the general web. If you are having difficulty, please contact Bill Ward (William.W.Ward @MedStar.net) or me (Ronald.Charlton@jax.ufl.edu). For your password use a five-digit number. Do not use leading zeros. For more information, click on the "New Login Procedure" link found on the main "News and Updates" page.

The "About ASHI" area is a great place to learn some "basics" about our organization. The area includes a brief history of ASHI, our mission and vision statements, a glossary of histocompatibility and immunology terminology and some good basic patient information about what we do. There is also a description of the origin for the various awards given at the Annual meeting along with a listing of past recipients. For new members and old, the "About ASHI" area is one that you might want to review or to refer someone else who is "just interested."

The "Accreditation" area is a treasure trove of information for new laboratories and existing laboratories. Here you can find out how the board is set up (history), who is your commissioner and how to contact her/him (review board). You can get a copy or portion of an application for a new lab or renewal (accreditation application). This area also has some sub-pages that list the inspection cycles, outline the accreditation process and describe the appeals process. If you are an inspector needing updating or want to be an inspector, there is a schedule of the upcoming inspector training sessions (Inspector Training). The "Accredited Laboratories" section lists all accredited laboratories, their lab numbers and the areas in which they are accredited and in "Inspection Schedule" you can see when your laboratory is to be inspected. For information about how the Accreditation Review Board (ARB) is structured and how it functions, with a copy of their operation manual, look in "Policy Update" and "Operations Manual." Those individuals who want to be laboratory directors or if you have a question about qualifications for directors, how they are evaluated and by whom, look in "Director Training Review." If you have a question or comment about the accreditation process, you can send an e-mail to the ARB at the "Questions and Feedback" page. There is also a search opportunity on the "Accreditation" page if you don't know where to look for the answer to a question.

From time to time we all have questions about Standards, By Laws and how to get hold of an officer or committee chair or member. These areas are covered in the "ASHI Governance" area. This page includes a link to the directory listing for our new ASHI Executive Office.

I hope these brief surveys of different areas of the web site help with your use of this communications tool. Please contact Rick Charlton or Bill Ward with suggestions for changes or additions that you would like to see for the web page.

Improve your typing skills.

The Most Comprehensive Range of Liquid Handling and Disposable Products Available for All Tissue Typing Methods

The First and Still the Best in Tissue Typing Products

Robbins continues to expand its product line for serological-based typing methods. From our new line of typing trays and syringe products to our automated serum and oil dispensers, Robbins sets the quality standard for tissue typing.

All PCR-based DNA Typing Methods Start with Robbins Products

Join the industry leader in PCR plasticware and liquid handling equipment. Choose from Strip-Ease® PCR tubes, CyclePlate®-384, -96, -48, -32 and -24 well thin wall PCR plates or our Hydra®-96 and -384 Channel Microdispenser for automated dispensing of PCR reagents and templates.

Keep Your Typing Skills in Peak Condition...

Contact Robbins at the numbers below to receive brochures for Tissue Typing and PCR products or visit our web site at *www.robsci.com*

Apogent Discoveries

©2002 Robbins Scientific Corporation; All Rights Reserved.

Robbins®

ROBBINS SCIENTIFIC CORPORATION
1250 Elko Drive Sunnyvale, CA 94089-2213
(408) 734-8500 (800) 752-8585 FAX (408) 734-0300
e-mail: custserv@robsci.com Internet: www.robsci.com

Accreditation News
April ARB Meeting

Geof Land, PhD, HCLD

Co-Chair Meeting

There were two major topics of discussion 1) possible replacements for the positions of Junior Co-Chair and new Commissioners to replace the ones rotating off and 2) the Inspector Training Subcommittee Chair (see below). A list was drawn up for prospective Co-Chair and Commissioner candidates and will be circulated to the ARB. The final slate will be voted upon at the August meeting, with subsequent publication in the next ASHI quarterly article.

Additional cost cutting measures were explored: 1) Karen Feder from the executive office will be charged with checking as to whether Saturday stay-over fares are necessary in certain areas, enabling the ARB meeting to be shortened without being penalized by an expensive airline fare. 2) Melissa McElroy is to determine the number of on-site inspections per cycle, so that a proactive approach to may be instituted in planning the most cost-effective approach to evaluating these packets.

Carol Pancoska shared with the Co-Chairs a draft of the proposed UNOS guidelines for determining the appropriate number of laboratories a director can direct. These will be discussed in depth as soon as they are finalized and adopted by UNOS.

ARB Meeting

There were a total of 37 interim laboratories in Cycle 3A. A total of seven labs had issues pending from the pre-meeting conference call. These labs were discussed in detail at the meeting, with one laboratory requiring a focused inspection.

There were a total of 24 on-site applications reviewed at the meeting. Also reviewed in this category were four laboratories needing ad-hoc, off cycle inspections. All of the laboratories were approved and granted reaccreditation. However there were 13 laboratories with minor pending issues and two laboratories with serious issues relating to past deficiencies. There will be a focused re-inspection of these two laboratories within six months. Three laboratories required an out of cycle review as a follow up from the last cycle.

New Items

Policy Review and Modification - 16 policies were reviewed and discussed with approved amendments/revisions to 12, and two with pending issues, which were subsequently resolved in a conference call. These will be made available to the membership on the ARB website and in our Operations Manual.

Web Document Presentation - Norm Skog, president of Amedco Inc., and John Hart presented two documents (Commissioner's Summary Report and the Inspector's Checklist) in a new proposed format connected through the Internet. The purpose is to create greater efficiency in reporting information by making it electronically available and interactive. John Hart presented the Inspector's Checklist Web Version at the Inspector's Training Workshop in Baltimore, April 15, 2002.

There were several new "mini" - checklists adopted by the ARB at the April meeting, which deal with some specialized areas of accreditation

1. Transfusion Support - Carol Pancoska presented this new checklist. A disclaimer will be added specifying that these are proposed guidelines pending approval by CMS.
2. Monitoring for Engraftment (ME) - Kevin Harrell and Marilyn Pollack presented the ME document and it was approved by the ARB. These are to be included in upcoming inspection packets for laboratories requiring ME. A disclaimer will be added on the checklist, specifying that these are the proposed guidelines pending approval by CMS.
3. Proficiency Results Summary Form* - Gary Teresi presented a revised and updated form. ASHI is in the process of talking with the new PT vendor to see if maybe some of the calculations the laboratory is required to make with the current system may be done automatically by the vendor.

The above examples are forerunners of what the ARB hopes will be the development of the ability to generate custom checklists to fit the actual services a member laboratory offers, rather than the "one-size-fits-all" approach we have now. This, hopefully, will be one of the advantages of the interactive, web-based protocols that Norm Skogs and John Hart are developing.

Inspector's Training Program

New Inspector Training Chair

As discussed in the previous issue of the *Quarterly*, Kathie Muto is stepping down as Chair of the Inspector Training Committee. Kathie has done an incredible job not only in the de novo development of the training program but also the development of a quality improvement program to monitor it. The success of both ventures can be measured by the number of excellent and conscientious new inspectors that we now have. Kathie deserves the thanks of the entire Society for the tremendous amount of energy and dedication she has placed into this area of accreditation. In addition, Dod Stewart and John Hart also deserve the Society's thanks for their efforts in helping Kathie put on these workshops.

Liz Trimble, soon to be rotating off of the ARB, has volunteered to step into the role as the inspector training chair and was approved by the board. She will begin training with Kathie Muto at the Regional Workshops and at the Annual meeting.

Workshops:

The full-day Inspector Workshop scheduled for the National Meeting in October has been cancelled. Instead a half-day update session will be offered. This was done in order that the ARB can meet its commitment to qualify all of the current trainees.

In order to help the ARB meet this commitment we would ask that directors and supervisors consider permitting any trainee Inspectors they may have working in their laboratory to participate as a trainee during their own laboratory's on-site inspection.

Inspector's Corner

There have been concerns expressed about the number of current ARB members who did inspections this last cycle. Inspectors for this cycle were chosen immediately after the tragedy at the New York World Trade Center. This led directly to having very few volunteers for both national and international inspections as well as several cancellations by inspectors who had already been chosen. Consequently, inspectors had to be found on an emergency basis and ARB members stepped in to fill the void. However, because of those expressed concerns and the need for the ARB members to focus on accreditation and not inspections we've instituted two policies.

1. While an individual is a member of the ARB (Commissioner, Co-Chair, or Program Director), s/he is not required to maintain the minimum number of inspections per year necessary to remain a qualified inspector. Service on the ARB will be deemed equivalent to doing inspections.
2. Foreign Inspections - a modification of the current policy.
    a. Inspectors for foreign labs are to be drawn from two main areas of talent: 1) past ARB members; to include past commissioners, co-chairs, program directors, and advisory board members. These individuals must not be currently serving in any of these capacities. A list of eligible individuals will be maintained in the accreditation office. 2) A pool of inspectors who have proven over time that they have exemplary diplomatic and scientific skills will also be deemed eligible for foreign inspections.
    b. The advisory board will be provided these two lists at the start of each cycle and will choose the inspectors for foreign laboratories.
    c. Only in extreme emergency situations will a member of the current ARB or advisory board be asked to inspect a foreign laboratory. An exception might be if an ARB/advisory board member was already scheduled for a trip or meeting in a country where a laboratory or laboratories were due an inspection. Then, for reasons of cost-effectiveness, that person would be considered.

Director Training Review Committee (DTR)

Dr. Sandra Helman reported the following for the DTR committee: Three new director portfolios have been completed and approved (Dr. Tom Ellis, Dr. David Horio and Dr. Ian Gourley). Drs. Ellis and Horio have had successful interviews; Dr. Gourley's interview is still pending at this date. Nine directors have submitted ad hoc portfolios for adding new categories and technologies, eight of nine have been approved at this date. There are five portfolios, two new directors and three additions of categories/ technologies, currently awaiting assignment of a reviewer.

A Director Training Program has been approved for the University of New Mexico, under the direction of Thomas Williams, MD.

Dr. Helman also reports numerous calls concerning the addition of new categories and technologies, which just haven't been formalized to the submission stage as yet.

The DTR Committee has performed yeoman service for the ARB this past quarter and is to be congratulated for the rapid and accurate evaluations of these portfolios and programs. Their activities serve to underscore the necessity of having good and conscientious reviewers on this committee. As the fields of Histocompatibility and Transplant Immunology broaden and with the constant development of new technologies, there will be an increasing need for members for this committee. If you would like to be a part of this dynamic and extremely important committee, then please contact Dr. Sandra Helman at the Histocompatibility/Immunology Laboratory, MCG Health System (706/721-3311; shelmanmail.mcg.edu)

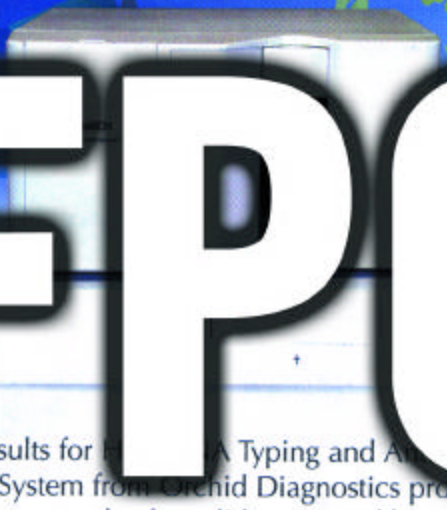
Recent Guideline Updates

The new UNOS standards have been published and are in effect. There are several standards, which now differ considerably from their current ASHI counterpart. In order to help ASHI become immediately compliant, Marilyn Pollack and Carol Pancoska helped to draft some interpretive guidelines for the ARB inspection checklists to accommodate those changes. These guidelines were accepted by the ARB and will be sent immediately to member laboratories, along with a copy of the UNOS standards. Laboratories will be inspected for compliance with these standards beginning with this cycle.

LIFE MATCH™

HLA/DNA Typing and Antibody Detection

SIMPLIFIED



FPO

Achieve quick, a[illegible] results for H[illegible]A Typing and A[illegible]ion with one instrument. The LifeMatch System from Orchid Diagnostics provides an automated, easy-to-operate method to analyze samples for solid organ and bone marrow transplantation. With the LifeMatch System you'll see:

FAST RESULTS

- Analysis time of one minute per reaction
- One reaction with over 50 different oligonucleotide probes or HLA antigens
- Compensation between red and green fluorescence unnecessary

CONVENIENT AND FLEXIBLE TESTING

- Runs 1 to 96 samples at a time
- Multiple reactions in a single tube reduces consumables

HIGH SENSITIVITY

- Flow cytometry based technology

Simplify your testing methods. Achieve more in less time. Contact Orchid Diagnostics today to learn more about the LifeMatch System and available tests.

ORCHID

ORCHID DIAGNOSTICS
550 WEST AVENUE
STAMFORD, CT 06902
1-800-543-3263 • Fax: 203-328-9599
www.orchid.com