International Immunopharmacology 13 (2012) 483–489

Contents lists available at SciVerse ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

The immune-modulator AS101 reduces anti-HLA antibodies in sera of sensitized patients: A structural approach

Moshe Israeli [a,c,*,1], Benjamin Sredni [c,2], Eitan Mor [b,d], Chana Zakai [a], Boaz Tadmor [b,e], Tirza Klein [a,b]

[a] Tissue Typing Laboratory, Rabin Medical Center, Petach-Tikva, Israel
[b] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[c] CAIR Institute, Goodman Faculty of Life-Sciences, Bar-Ilan University, Ramat-Gan, Israel
[d] Organ Transplantation Department, Rabin Medical Center, Petach-Tikva, Israel
[e] Central Research Authority, Rabin Medical Center, Petach-Tikva, Israel, Israel

ARTICLE INFO

Article history:
Received 18 March 2012
Received in revised form 22 May 2012
Accepted 25 May 2012
Available online 7 June 2012

Keywords:
Immune-modulator
AS101
Anti-HLA antibodies
Epitope
HLA Matchmaker
Transplantation

ABSTRACT

Background: Significant efforts are dedicated to identification of agents that eliminate anti-HLA antibodies (Ab) from sera of transplant candidates. Antibody titer following *in vitro* incubation of sera with desensitizing agents has shown to reflect the probability that a patient would benefit from clinical de-sensitization protocols. AS101 is a non-toxic, synthetic, organic tellurium compound. The aim of this research was to assess the ability of AS101 to reduce anti-HLA Abs and to identify patients likely to benefit from this effect.
Methods: Sera of sensitized patients awaiting transplant were incubated in the presence of AS101. Measured mean fluorescence intensity (MFI) represents reactivity of anti HLA Abs in the serum, as detected by the Luminex platform. The repertoire of HLA antigen epitopes was recognized using HLA Matchmaker software.
Results: AS101 Incubation caused a significant Ab titer decrease in approximately two thirds of the samples. The median Class I and II MFI decrease among the responding samples was 16.7% and 14%, respectively ($p<0.05$). HLA Matchmaker analysis of the patients' class I epitope sequences revealed apparent amino-acid differences between the patterns of the responding and non-responding patients.
Conclusion: In vitro incubation of sera in the presence of AS101 causes a decrease in the anti-HLA Ab's reactivity in several patient samples. Sera most likely to demonstrate this effect are characterized by a moderate MFI level and a distinct antibody reactivity pattern specific for defined HLA antigen epitopes. These results support further investigation of AS101 as a potential agent for desensitization of humoral reactivity prior to transplantation.

1. Introduction

Donor specific antibodies directed against HLA antigens of the allograft present a significant obstacle to successful organ transplantation into highly-sensitized patients [1–3]. Removal of donor specific anti-HLA antibodies prior to transplant and elimination of their *de novo* appearance after it, contributes to graft survival and to prevention of graft rejection [4–6].

Continuing effort is dedicated to identification of agents that can bring about a decrease in anti-HLA Abs titer [7,8]. In vitro incubation of patient sera with desensitizing agents has been shown to reflect the probability that a patient would benefit from such de-sensitization protocols [9–11].

AS101 (ammonium trichloro [dioxoethylene-o,o] tellurate) is a low molecular weight, synthetic, organic tellurate compound developed in the laboratories of Prof. B. Sredni and Prof. M. Albeck at Bar Ilan University in Israel. Its development was based on the anti-tumoral agent Cisplatin, by the substitution of the tellurium for the central platinum atom; it is soluble in organic solvents, but only slightly in water [12].

AS101 was found to possess anti-viral and anti-parasitic properties owing to its immunomodulatory activity [13,14]. The antitumoral effect demonstrated by this compound is derived from its immunomodulatory activity on the cells of the immune system, as well as its direct effect on cancer cells [15–18]. Toxicity tests showed that at the effective immunomodulatory dose, AS101 presents no negative side effects [19]. The *in-vivo* immunomodulatory activity of AS101 together with its anti-tumoral activity [20] has prompted examination of its efficacy in various clinical trials. Previous publications have shown that a part of AS101's biological activity arises from its chemical interactions with cysteine thiol residues which may lead to conformational modifications of protein structure [21,22]. This indicated to the possibility that AS101 could possibly have an immunomodulatory influence on the structure

Abbreviations: aa, Amino acid; Ab, Antibody; CREG, cross reactive groups; HLA, Human leukocyte antigen; IVIg, Intra-venous Immuno-globulin; MFI, Median Fluorescence Intensity; mg, miligram; mL, Milliliter; PP, Plasmapheresis; STD, Standard Deviation.

* Corresponding author at: Tissue Typing Laboratory, Rabin Medical Center (Beilinson campus), Zabotinski Rd., Petach-Tikva, 49100, Israel. Tel.: +972 3 9376747, +972 77 7654607, +972 52 8654607(mobile); fax: +972 3 9376786.
E-mail addresses: israelimoshe@yahoo.com, mosheis@clalit.org.il (M. Israeli).

[1] This work was conducted as part of the Ph.D. thesis requirements of Moshe Israeli at Bar-Ilan University.

[2] This author contributed equally to this study as the first author.

1567-5769/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.intimp.2012.05.019

and function of Abs, namely anti-HLA Ab's. We therefore initiated an *in-vitro* study aimed to evaluate AS101's ability to reduce the titer of anti-HLA Ab's in the sera of highly-sensitized patients awaiting an organ transplant.

2. Materials and methods

2.1. Patients and samples

Samples used consisted of 102 sera collected from sensitized patients (PRA above 80%) awaiting organ transplantation or immediately following transplant surgery. All patients were between the ages of 18 and 80. Of the samples, 51 sera were collected from male patients and 51 sera from female patients. Sensitized patients were defined as transplant candidates, in whom presence of anti-HLA antibodies was detected in their serum at least once, using one of the standard methods (such as ELISA or Luminex screen). 86 and 73 sera were sensitized against HLA Class I and HLA Class II, respectively. This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local ERB of the Rabin Medical Center.

2.2. Sera incubation

Sera were incubated for 3 hours at room temperature on a Titramax 101 orbital shaker (Heidolph Instruments, Germany) at a speed of 300–400 rpm. For *in-vitro* IVIg incubation Omr-IgG-am infusion solution was used, which was stored at 4 °C in the dark (manufactured by Omrix Biopharmaceuticals ltd, Israel). AS101 was supplied by M. Albeck from the Department of Chemistry at Bar-Ilan University in Israel, in a 15 mg/mL solution in PBS, pH 7.4, and maintained at 4 °C. Incubation in the presence of PBS (Biological Industries, Bet-Haemek, Israel) was used as the control.

2.3. Detection of anti-HLA antibodies

Anti HLA antibodies were detected using the LIFECODES LifeScreen Deluxe, ID and single antigen assays, which are qualitative bead-based immunoassays used to detect IgG antibodies to HLA Class I and Class II (Genprobe Transplant Diagnostics, Inc., Connecticut USA). The assays were performed according to the manufacturer's directions. Briefly, an aliquot of the beads was incubated with a small volume of test serum sample. The sensitized beads were then washed to remove unbound antibody. An anti-Human IgG antibody conjugated to phycoerythrin was then added. After incubation, the test sample was diluted and analyzed on the Luminex-100 instrument (Luminex Corporation, Texas USA). The signal intensity from each bead was compared to the signal intensity of a negative control bead included in the preparation. Each HLA-antigen coated beads blend includes four positive control beads. In each Luminex run we incorporated a positive control serum obtained from individuals shown to be alloimmunized to HLA antigens. And also a negative control serum obtained from individuals known to have no antibodies to HLA antigens, all supplied by the manufacturer. A positive Ab reduction response was defined as any event where serum incubation in the presence of an immune-modulator brought about a reduction of more than 5% in the measured MFI in comparison to the control. In order to avoid the problem of inter-assay fluorescence variation and inter-assay controls - all MFI comparisons between a treated and untreated sample or of a sample under various treatments was only performed among measurements within the same assay run. In this study we avoided comparing MFI levels that were obtained for the same sample in separate assay runs in the Luminex platform.

2.4. Antigen structure analysis

The repertoire of polymorphic amino acid (aa) residues on HLA antigen epitopes was recognized using HLA Matchmaker software version 2.1 (Duquesnoy RJ, UPMC, USA). HLA Matchmaker implements a theoretical algorithm that considers that each HLA antigen represents a collection of aa patches in antibody-accessible positions. These so-called eplets represent key elements of functional epitopes that can elicit HLA-specific alloantibody responses. The software is capable of presenting the sequence of aa's in these epitopes of a specific patient based on his HLA antigen typing. Color analysis of amino acid sequences is based on the RasMol amino color scheme, version 2.6, Molecular Graphics Visualization Tool (Glaxo Wellcome Research & Development, Hertfordshire, UK).

2.5. Statistical analysis

Statistical analysis was performed using SPSS 15.00 software (Illinois, USA). The “Paired sample *t*-test”, and “Independent sample *t*-test” were used as indicated. Fisher's exact test was used for categorical data (responders/non-responders) to examine the significance of the association (contingency) between the two kinds of classification.

3. Results

3.1. Sera incubation in the presence of AS101 decreases measured MFI levels

Sera incubation in the presence of AS101 (concentration 4 microgram/mL) caused a decrease of more than 5% in the measured MFI levels in 54 of the 86 samples that were sensitized against HLA Class I antigens and in 51 of the 73 sera that presented Ab's against HLA Class II. The median Class I MFI decrease among the responding samples was of 16.7% (STD ± 10.8%, $p<0.05$). Similar results were observed among sera that were sensitized against HLA Class II antigens where an average MFI decrease of 14% (STD ± 9.7%) was measured in the responding samples (Fig. 1a).

3.2. Comparison and combination of two immunomodulators: AS101 and IVIg

In a comparative experiment, 10 sera of highly sensitized patients awaiting a renal transplant (PRA Class I & Class II > 80%) were incubated *in vitro* in the presence of either IVIg or AS101. AS101 caused an average decrease of 16% in the measured MFI levels of anti HLA Class I Ab's compared to the PBS treated control, in which 5 of the 10 sera showed a reduction in MFI levels. IVIg incubation brought about an average decrease of 13% in the measured MFI levels of anti HLA Class I Ab's compared to the control, where 4 of the 10 sera showed an advantageous response. Furthermore, incubation of sera in the presence of IVIg and AS101 led to an average decrease of 5% and 15%, respectively, in measured MFI levels of anti HLA Class II Ab's. The effect of IVIg effect was visible in 4/10 sera while AS101 effect was identified in 6/10 samples (Fig. 1b). The responding sera to AS101 and to IVIg did not necessarily overlap and several samples showed a response to only one of the two immunomodulators.

In a subsequent experiment, 12 sera were incubated in the presence of IVIg, in combination or without AS101. Measured MFI levels measured in samples incubated in the presence of IVIg alone or in the combined presence of both immune-modulators were lower than the control by 13% and 16%, respectively. In additional experiments three sera collected from patients who were treated with IVIg + Plasmapheresis (PP) *in vivo* as part of the clinical protocol were incubated in the presence of AS101. The range of MFI decrease following *in vitro* AS101 treatment was 8–28% in comparison to the sera not treated with AS101.

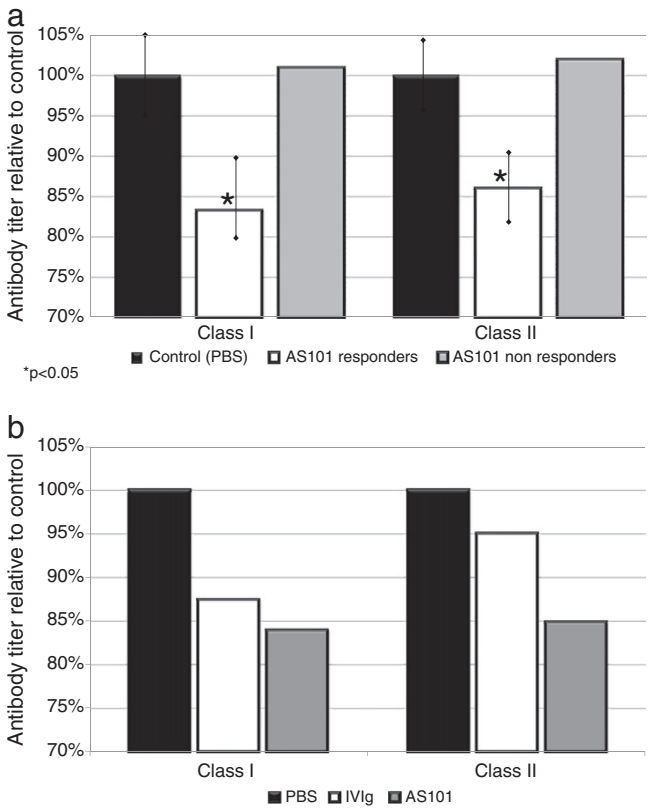

Fig. 1. Incubation in the presence of AS101 causes a decrease in the measured MFI level of anti-HLA Ab's in selected sera. (a) Sera of sensitized patients awaiting transplant were incubated (3 hours, room temperature) in the presence of AS101 (concentration 4 microgram/mL). Incubation in the presence of PBS is considered as control. An Ab reactivity decrease was perceptible in 54 of the 86 samples sensitized against HLA Class I (median decrease 16.7%) and in 51 of the 73 sera sensitized against HLA Class II (median decrease 14%). (b) In a comparative experiment sera were incubated (3 hours, room temperature) in the presence of AS101 or of IVIg solution. The in vitro effect of the two immunomodulators on the Ab titer in the sera was similar.

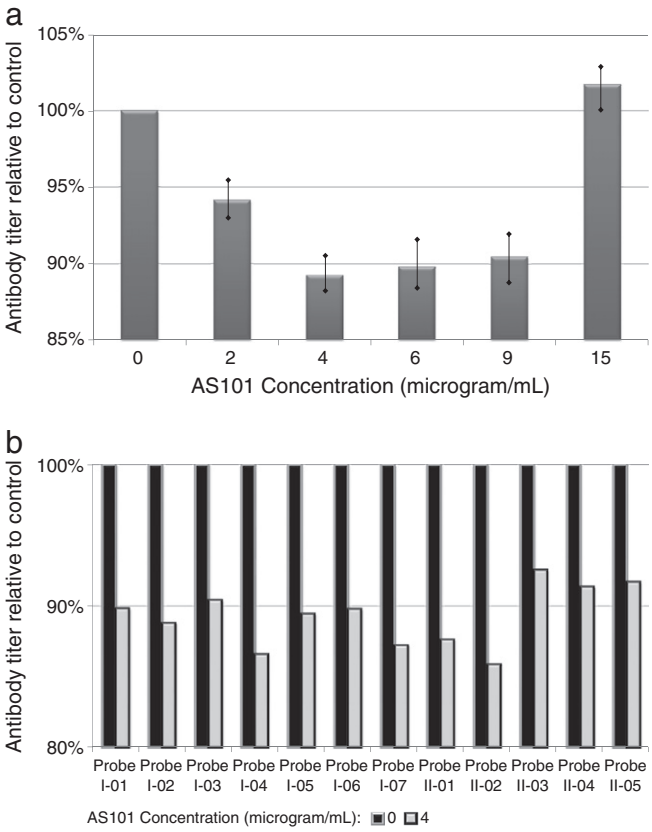

Fig. 2. Dose response curve to incubation of sera in the presence of AS101. (a) Each serum was tested in parallel with a series of gradient AS101 concentrations (2, 4, 6, 9, 15 microgram/mL). The 4 microgram/mL concentration was found to generate the most substantial effect. (b) Separate analysis of CREG-enriched beads shows that AS10's1 effect on antibodies of different CREG's was not identical, thereby implying that the MFI decrease is not a measurement artifact but rather is influenced by structural traits of the reacting molecules.

3.3. Specific conditions are required for the MFI level reduction by AS101

In order to find which AS101 concentration produces an optimal effect in reduction of MFI levels we tested each sample with in parallel with a gradient series of AS101 concentrations. We found that incubation with an AS101 concentration of 4 μg/mL induces the strongest effect (Fig. 2a). Analyzing the fluorescence pattern of the various CREG-enriched beads showed that AS10's1 effect on antibodies of different CREG's was not uniform, as shown in Fig. 2b. Different CREG-designated antibodies respond differently and reproducibly to incubation in the presence of AS101, thereby indicating that the reduction of anti-HLA Ab's is not a measurement artifact, but rather a phenomena caused by AS101's influence on the antibodies.

3.4. Responding sera are characterized by a moderate MFI level

Among the 64 sera that were tested for Class I Ab's using the Luminex screen assay, the responding sera had a pre-treatment median MFI level that was significantly lower than the non-responding sera: 4169 vs. 13,024, respectively ($p<0.05$). A similar observation was noticed among the 57 sera that were screened for Class II Ab's where responding and non-responding sera had a median pre-treatment MFI level of 4231 vs. 13,092, respectively ($p<0.05$) (Fig. 3). Fig. 4 provides a schematic overview of the Ab reactivity pattern of the responding vs. non-responding sera, showing that there is no apparent difference between the two groups with regard to the antibody specificities but rather a difference in the level of sensitization of the sera. The lower section of the figure includes more red colored blocks indicating that many Ab's exhibit an MFI above 10,000.

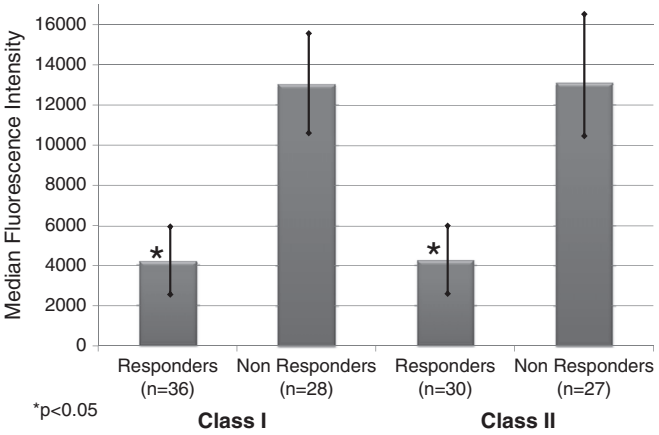

Fig. 3. Sera with a moderate MFI level are more responsive to the AS101 effect. Responding sera are characterized by a moderate median MFI level (HLA Class I: 4169 ; HLA Class II: 4231), which is significantly lower than the high median MFI levels which typify the non-responding sera (HLA Class I: 13,024; HLA Class II: 13,092). Vertical lines represent 95% confidence intervals.

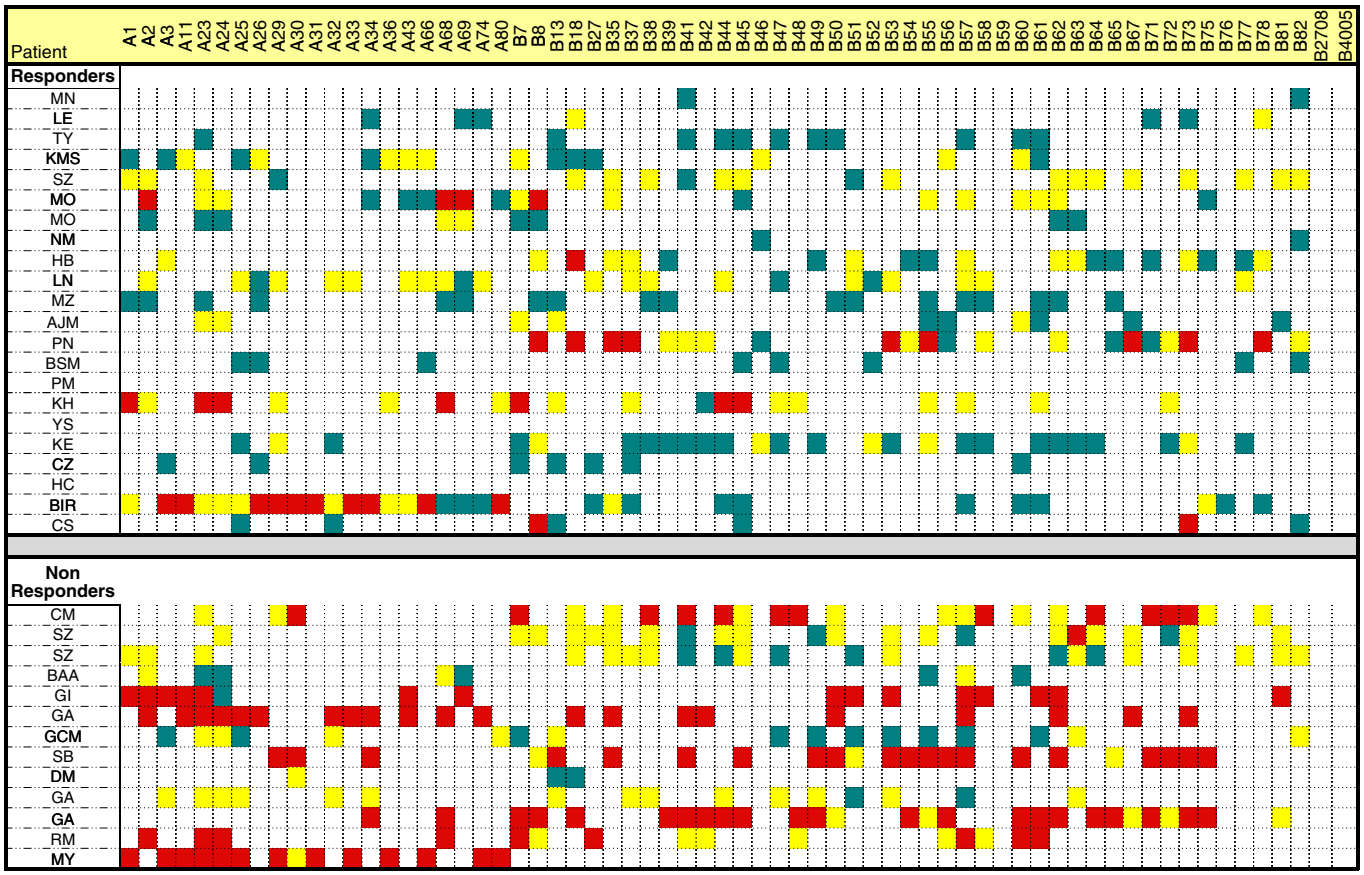

Fig. 4. Schematic overview of the humoral sensitization pattern of responding vs. non-responding samples. Each horizontal row represents the antibody repertoire of a single patient. Red, yellow and green blocks represent the presence of an antibody of that specificity with an MFI above 10,000, or 10,000 > MFI > 4,000, or MFI < 4,000, respectively. The responsing sera (top panel) display more green and yellow blocks in comparison to the non-responding sera (bottom panel) which are represented by more red blocks. No noticeable association between the HLA antigen specificity of the antibodies in the serum and the pattern of response to AS101 treatment.

3.5. Responding sera present a distinct structurally defined HLA antigen epitope pattern

In order to find a structural characteristic, that could differentiate between the Ab repertoire of the responding and non-responding sera we compared the HLA antigen epitope sequences of the patients, as determined by the HLA Matchmaker software. The software was provided with the HLA Class I antigen typing of each patient. The software generated an output displaying the sequence of aa's located in the antibody accessible epitopes of the HLA antigens of each patient. The sequence of aa's on the antibody-generating epitopes of a patient influences the anti-HLA antibody panel that that person is capable of producing, as a person will not produce antibodies against his own epitopes. Thus, analysis of the epitope pattern of a patient or moreover of a group of patients can indicate to a shared antibody reactivity pattern among these patients. Our analysis of class I epitope sequences revealed 16 statistically significant amino-acid sequence disparities between the patients presenting the responding and non-responding sera (Fig. 5, Fisher's exact test). Identification of epitope disparities that are coherent across the HLA antigens of the responding and non-responding patients indicates to a possible structural distinction of the Ab's repertoire among the two sera groups that were analyzed in this study.

4. Discussion

Presence of donor specific anti HLA antibodies in the serum of prospective transplant recipients is one of the major causes for transplant delay and of poor transplant outcome and therefore great effort has been devoted to finding ways to overcome this immunologic barrier [23–25]. Leading strategies implemented for overcoming the anti-HLA Ab barrier include logistic solutions such as donor exchange and unique allocation systems [26] or therapeutic methods of desensitization, many of which include utilization of IVIg as well as other immune-modulators [27,28]. Knowing the immune-modulatory characteristics of the AS101 compound and its chemical effect on thiols and disulfide bonds, we initiated this research, intended to explore the compound's effect on sera of sensitized patients awaiting transplant. The results of the current study show that incubation in the presence of AS101 causes a perceptible decrease in the reactivity of anti-HLA Class I and Class II antibodies in approximately two thirds of the tested sera. The remaining sera were not affected by incubation in the presence of AS101. Our analysis considered a positive response to the AS101 incubation in samples where an MFI decrease of at least 5% was detected, as was also implemented in previous publications in which the *in vitro* effects of IVIg were studied [10,29].

AS101 caused a reduction in Ab reactivity even in serum samples that benefited from *in-vitro* treatment with IVIg, suggesting a potential beneficial additive effect of using both immune-modulators in combination. Surprisingly, there were sera which benefited from AS101 treatment although they were not affected by *in vitro* IVIg treatment. Furthermore, AS101 also brought about a decrease in the Ab reactivity of patients who were clinically treated *in vivo* with IVIg + PP, suggesting that AS101 treatment may possibly have additional beneficial effects in patients undergoing common desensitization treatments.

In vitro reduction of anti-HLA antibodies reactivity has been shown to serve as a possible indicator to the likelihood that de-sensitization

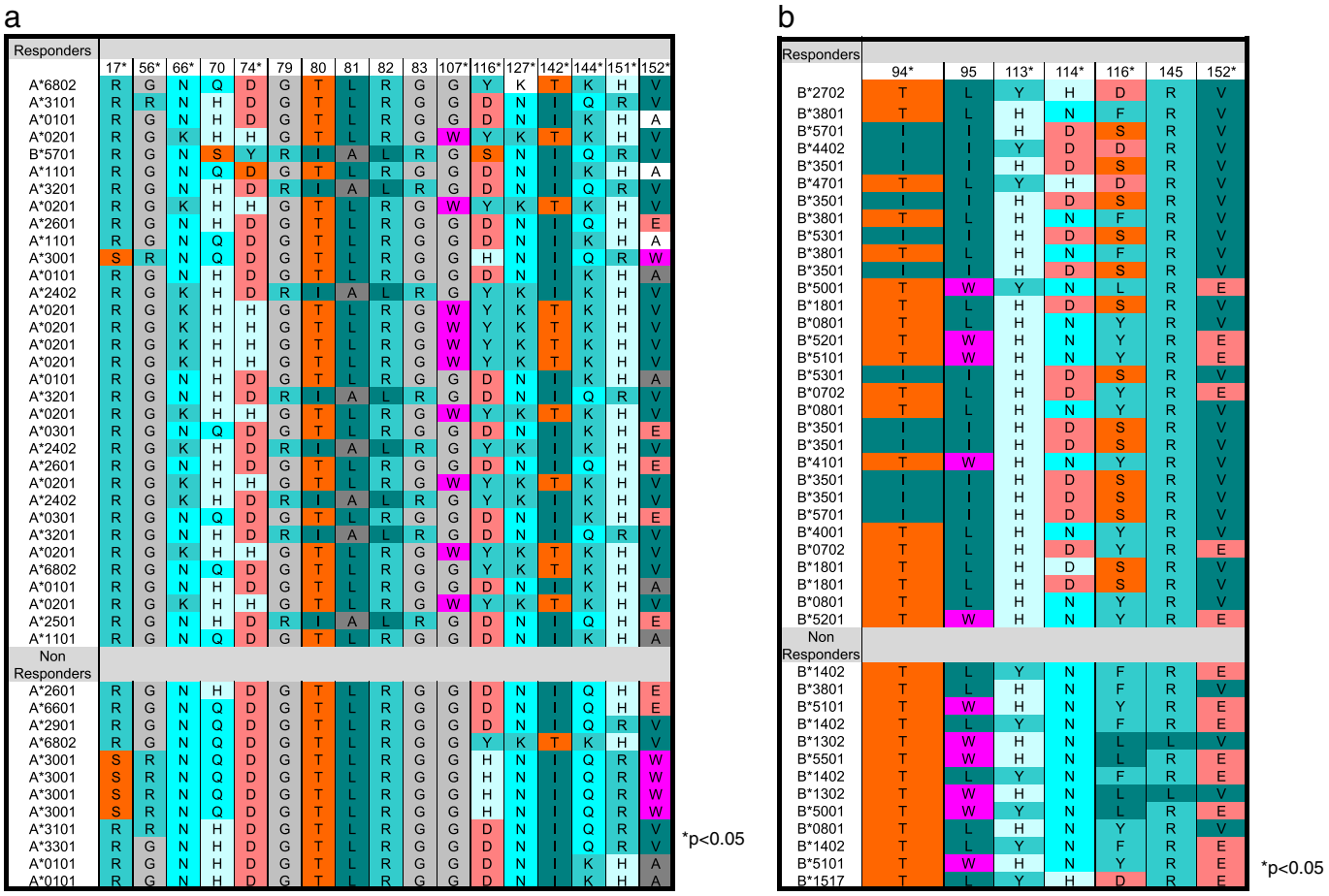

Fig. 5. Epitope sequence disparities between AS101 responding and non-responding samples. Epitope sequences of HLA Class I antigens were determined using the HLA-Matchmaker software for A* alleles (a) and for B* alleles (b). Amino acids are colored according to RasMol color scheme (Glaxo Wellcome Research & Development, Hertfordshire, UK). Numbers in the top row indicate the position of the amino acid in the protein sequence.

treatment could be beneficial for the patient [11]. The current study demonstrating the beneficial consequence of *in-vitro* AS101 incubation on the Ab reactivity of sensitized sera, with or without IVIg, suggests further analysis of the optimal conditions required for this effect. It is important to emphasize that despite its utilization in multiple phase II clinical trials in various clinical settings, AS101 has never been used *in vivo* for the purpose of desensitization treatment of organ transplant candidates. This present study provides a maiden opportunity for 'proof of concept' experimentation where the current *in vitro* findings could provide a basis for further *in vivo* experimentation.

The AS101 incubation outcome of the responding sera exhibited a 'bell-shaped' dose effect where very low and very high AS101 concentrations caused an inferior effect in comparison to a moderate concentration of 4 microgram/mL. Similar 'bell shaped' dose effects were observed in other investigational settings where the activity of AS101 as an immune-modulator was studied [30,31], thus indicating that our current observation could also be attributed to the compound's acknowledged immunomodulatory and chemical characteristics.

The non-uniform effect of AS101 on Ab's of various CREG's as depicted in Fig. 2b indicates that the reduction of the anti-HLA antibodies reactivity as a result of the interaction with AS101 is probably dependant on structural traits of the immunoglobulin rather than a measurement artifact. The difference in the effect of AS101 incubation on responding and non-responding sera motivated us to search for a noticeable characteristic that would differentiate between the two sera groups. Fig. 4 illustrates that there is no apparent difference between the responding and the non-responding sera with regard to the antibody specificity. Sera from both groups contain antibodies directed against a very wide range of HLA antigen specificities. It was noted that the responding sera have a moderate MFI level, where most of the detected antibodies were measured at an MFI that is bellow 10,000, as presented in Figs. 3 and 4. Similar observations have been reported in the literature in regard to IVIg and other desensitization protocols, where distinction between patients who responded or who did not respond to treatment was in apparent linkage with high Ab titer [32,33].

Accumulated evidence suggests that much of the biological activity of the AS101 compound is related to its chemical interactions with cysteine thiol residues. The Te(+4)-thiol chemical bond may lead to conformational change or disulfide bond formation in a specific protein, possibly resulting in the loss of its biological activity, if the thiol residue is essential for that function. Indeed, previous publications have demonstrated that AS101 and other Te(+4)-compounds specifically inactivate cysteine proteases, while exhibiting no effect on the other families of serine, aspartic and metallo-proteases, in good agreement with the predictions of their unique Te(+4)-thiol chemistry [21,22,34].

We therefore sought to identify a structural characteristic of the anti-HLA Ab's that would differentiate between the responding and non-responding sera, which could serve as a starting point for exploration of the distinct chemical influence of the AS101 compound on the two sample groups. Identification of a structural trait of the anti-HLA Ab's that is dissimilar between the responding and non-responding groups could help identify the chemical nature of the interaction of AS101 with these antibodies. Similar explorations have found evidence that the *in vitro* effects of IVIg on anti-HLA Ab's also originate in

structural characteristics influencing the reaction between the IVIg immuno-modulator and the Ab's [29].

Multiple studies have set the basis for structural HLA matching between a prospective organ donor and a designated recipient based on comparison of their HLA antigen epitope repertoire [35–37]. The structural approach determines histocompatibility at the epitope rather than antigen level in terms of the humoral allo-immune response. This is achieved through comparison of the HLA epitopes of the donor and recipient, namely analysis of the polymorphic amino acid residues on the molecular surface of their HLA antigens. This comparison allows identification of potential donor–recipient pairs, which, in spite of their mismatched HLA antigens, can be considered compatible at the structural level, minimizing the risk for a humoral allo-response [38,39].

For the structural analysis of the anti-HLA Ab's repertoire of the responding and non-responding sera, we utilized the HLA-Matchmaker program. This Excel-based application performs structural HLA matching at the epitope level and analyzes antibody reactivity patterns specific for structurally defined epitopes.

When provided with a person's HLA antigen typing the software generates the sequence of aa's present on the Ab-recognized epitopes in the HLA antigens of that individual. It is accepted that an individual's own HLA antigens represent the repertoire of self-epitopes to which no antibodies can be made. Thus, analysis of polymorphic amino acid residues on HLA antigens of the AS101 responding and non-responding patients might provide insight into the potential anti-HLA Ab's structural repertoire of these distinct patient groups. Fig. 5 depicts HLA Class I amino acid residues that are disparate between the patients with the responding and non-responding sera. Several residues display merely a trend of dissimilarity between the two sample groups while other residues present a statistically significant difference between the HLA antigen epitopes of sera from the two groups. This structural distinction in the HLA antigen epitopes between the two groups could indicate that there are also unique structural characteristics of the antibody repertoires of the two groups.

Noticeable structural distinctions between the HLA antigen epitopes repertoire of the responding and non-responding samples would suggest that there are structural distinctions in the anti-HLA Ab's repertoire of the two sample groups in this current study. It is possible that this structural uniqueness of the responding or of the non-responding immunoglobulins is related to AS101's ability to chemically react with these molecules. Our findings that the AS101 effect manifests at an optimal incubation concentration of 4 microgram/mL and discriminates between immunoglobulins related to different CREG's support the likelihood that specific chemical conditions are needed for executing this effect most favorably. Interestingly, the effect of AS101 was not evident when sera were tested with single antigen coated beads (data not shown). A possible reason for this is the dissimilar chemical characteristics of the antibody-antigen coupling in the setting of single antigen coated beads, where the process of attaching recombinant antigens to the beads causes denaturation of the antigen molecules on the beads [40].

Future exact characterization of the structural antibody profile of AS101 responding and non-responding sera will allow greater understanding of the exact mechanism by which the compound influences the reactivity of anti-HLA antibody in the serum. Continuing studies are warranted in order to determine whether reduction of pre-transplant Ab reactivity levels using the immune-modulator AS101 could serve as a beneficial component of the de-sensitization procedures prior to transplantation, thereby improving the likelihood of better transplant outcome [41,4].

Acknowledgements

The authors wish to thank Prof. Rene Duquesnoy from University of Pittsburgh Medical Center for critical reading of the manuscript and for his wise recommendations.

This work was supported in part by The Safdié Institute for AIDS and Immunology Research; The Dave and Florence Muskovitz Chair in Cancer Research; The Dr Tovi Comet-Walerstein Cancer Research Program; The Rothschild Caesarea Foundation.

References

[1] Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 1969;280:735–9.
[2] Stastny P, Lavingia B, Fixler DE, Yancy CW, Ring WS. Antibodies against donor human leukocyte antigens and the outcome of cardiac allografts in adults and children. Transplantation 2007;84(6):738–45.
[3] Singh N, Djamali A, Lorentzen D, Pirsch JD, Leverson G, Neidlinger N, et al. Pretransplant donor-specific antibodies detected by single-antigen bead flow cytometry are associated with inferior kidney transplant outcomes. Transplantation 2010;90(10):1079–84.
[4] Everly MJ, Rebellato LM, Ozawa M, Briley KP, Catrou PG, Haisch CE, et al. Beyond histology: lowering human leukocyte antigen antibody to improve renal allograft survival in acute rejection. Transplantation 2010;89(8):962–7.
[5] Lefaucheur C, Suberbielle-Boissel C, Hill GS, Nochy D, Andrade J, Antoine C, et al. Clinical relevance of preformed HLA donor-specific antibodies in kidney transplantation. Am J Transplant 2008 Feb;8(2):324–31.
[6] Lachmann N, Terasaki PI, Budde K, Liefeldt L, Kahl A, Reinke P, et al. Anti-human leukocyte antigen and donor-specific antibodies detected by luminex post-transplant serve as biomarkers for chronic rejection of renal allografts. Transplantation 2009 May 27;87(10):1505–13.
[7] Glotz D, Haymann JP, Sansonetti N, Francois A, Menoyo-Calonge V, Bariety J, et al. Suppression of HLA-specific alloantibodies by high-dose intravenous immunoglobulins (IVIg). A potential tool for transplantation of immunized patients. Transplantation 1993;56:335.
[8] Jordan SC, Peng A, Vo AA. Therapeutic strategies in management of the highly HLA-sensitized and ABO-incompatible transplant recipients. Contrib Nephrol 2009;162:13–26.
[9] Jordan SC, Tyan DB. Intravenous gamma globulin (IVIG) inhibits lymphocytotoxic antibody *in vitro*. J Am Soc Nephrol 1991;2:803.
[10] Tyan DB, Li VA, Czer L, Trento A, Jordan SC. Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. Transplantation 1994;57:553–62.
[11] Jordan SC, Vo A, Bunnapradist S, Toyoda M, Peng A, Puliyanda D, et al. Intravenous gammaglobulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients. Transplantation 2003;76:631–6.
[12] Sredni B, Caspi RR, Klein A, Kalechman Y, Danziger Y, Ben Ya'akov M, et al. A new immunomodulating compound (AS-101) with potential therapeutic application. Nature 1987;330:173–6.
[13] Sredni B, Rosenthal-Galili Z, Michlin H, Sobelman D, Seger Y, Blagerman S, et al. Restoration of murine cytomegalovirus (MCMV) induced myelosuppression by AS101. Immunol Lett 1994;43:159–65.
[14] Rosenblatt-Bin H, Klein A, Sredni B. Antibabesial effect of the immunomodulator AS101 in mice: role of increased production of nitric oxide. Parasite Immunol 1996;18:297–306.
[15] Kalechman Y, Albeck M, Oron M, Sobelman D, Gurwith M, Horwith G, et al. Protective and restorative role of AS101 in combination with chemotherapy. Cancer Res 1991;51:1499–503.
[16] Kalechman Y, Albeck M, Sredni B. *In vivo* synergistic effect of the immunomodulator AS101 and the PKC inducer bryostatin. Cell Immunol 1992;143:143–53.
[17] Sredni B, Tichler T, Shani A, Catane R, Kaufman B, Strassmann G, et al. Predominance of TH1 response in tumor-bearing mice and cancer patients treated with AS101. J Natl Cancer Inst 1996;88:1276–84.
[18] Hayun M, Saida H, Albeck M, Peled A, Haran-Ghera N, Sredni B. Induction therapy in a multiple myeloma mouse model using a combination of AS101 and melphalan, and the activity of AS101 in a tumor microenvironment model. Exp Hematol 2009 May;37(5):593–603.
[19] Nyska A, Waner T, Pirak M, Albeck M, Sredni B. Toxicity study in rats of a tellurium based immunomodulating drug, AS-101: a potential drug for AIDS and cancer patients. Arch Toxicol 1989;63:386–93.
[20] Hayun M, Okun E, Hayun R, Gafter U, Albeck M, Longo DL, et al. Synergistic effect of AS101 and Bryostatin-1 on myeloid leukemia cell differentiation in vitro and in an animal model. Leukemia 2007 Jul;21(7):1504–13.
[21] Albeck A, Weitman H, Sredni B, Albeck M. Tellurium compounds: selective inhibition of cysteine proteases and model reaction with thiols. Inorg Chem 1998;37:1704–12.
[22] Frei GM, Lebenthal I, Albeck M, Albeck A, Sredni B. Neutral and positively charged thiols synergize the effect of the immunomodulator AS101 as a growth inhibitor of Jurkat cells, by increasing its uptake. Biochem Pharmacol 2007 Sep 1;74(5):712–22.
[23] Gloor J, Stegall MD. Sensitized renal transplant recipients: current protocols and future directions. Nat Rev Nephrol 2010;6(5):297–306.
[24] Zachary AA, Eng HS. Desensitization: achieving immune detente. Tissue Antigens 2011 Jan;77(1):3–8.
[25] Beimler JH, Susal C, Zeier M. Desensitization strategies enabling successful renal transplantation in highly sensitized patients. Clin Transplant 2006;20(Suppl. 17):7–12.

[26] Claas FH, Doxiadis II. Management of the highly sensitized patient. Curr Opin Immunol 2009 Oct;21(5):569–72.
[27] Vo AA, Lukovsky M, Toyoda M, Wang J, Reinsmoen NL, Lai CH, et al. Rituximab and intravenous immune globulin for desensitization during renal transplantation. N Engl J Med Jul 2008;359(3):242–51.
[28] Montgomery RA, Zachary AA, Racusen LC, Leffell MS, King KE, Burdick J, et al. Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. Transplantation 2000;70(6): 887–95.
[29] Wassmuth R, Hauser IA, Schuler K, Erxleben H, Arnold ML, Koelman CA, et al. Differential inhibitory effects of intravenous immunoglobulin preparations on HLA-alloantibodies in vitro. Transplantation 2001 May 27;71(10):1436–42.
[30] Frei GM, Kremer M, Hanschmann KM, Krause S, Albeck M, Sredni B, et al. Antitumour effects in mycosis fungoides of the immunomodulatory, tellurium-based compound, AS101. Br J Dermatol 2007;158:578–86.
[31] Sredni B, Xu RH, Albeck M, Gafter U, Gal R, Shani A, et al. The protective role of the immunomodulator AS101 against chemotherapy-induced alopecia studies on human and animal models. Int J Cancer 1996;65:97–103.
[32] Kimball PM, Owens A, Gibney E. Quantitation of alloantibody concentration can predict patient sensitivity to intravenous immunoglobulin desensitization. Transplantation 2007;84:1540–3.
[33] Stegall MD, Gloor J, Winters JL, Moore SB, Degoey S. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. Am J Transplant 2006 Feb;6(2):346–51.
[34] Sredni B, Geffen-Aricha R, Duan W, Albeck M, Shalit F, Lander HM, et al. Multifunctional tellurium molecule protects and restores dopaminergic neurons in Parkinson's disease models. FASEB J 2007;21:1870–83.
[35] Duquesnoy RJ. A structurally based approach to determine HLA compatibility at the humoral immune level. Hum Immunol 2006;67:847–62.
[36] Dankers MK, Witvliet MD, Roelen DL, de Lange P, Korfage N, Persijn GG, et al. The number of amino acid triplet differences between patient and donor is predictive for the antibody reactivity against mismatched human leukocyte antigens. Transplantation 2004;77(8):1236–9.
[37] Duquesnoy RJ, Marrari M, Mulder A, Claas FHJ, Mostecki J, Balazs I. Structural aspects of HLA Class I epitopes detected by human monoclonal antibodies. Hum Immunol 2012 Mar;73(3):267–77.
[38] Duquesnoy RJ. Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation. Curr Opin Immunol 2008;20(5):594–601.
[39] Duquesnoy RJ, Takemoto S, de Lange P, Doxiadis II, Schreuder GM, Persijn GG, et al. 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. Mar 27 2003;75(6):884–9.
[40] Poli F, Benazzi E, Innocente A, Nocco A, Cagni N, Gianatti A, et al. Heart transplantation with donor-specific antibodies directed toward denatured HLA-A*02:01: a case report. Hum Immunol 2011;72:1045–8.
[41] Fidler S, Irish A, Lim W, Ferrari P, Witt C, Christiansen F. The peak and sum MFI of the single antigen beads (SAB) of donor specific anti-hla antibodies (DSA) at the time of transplantation in renal transplant recipients (RTR) associate with the risk of c4d^{+} rejection. Transplantation 2010;90:528.