Immunosuppression

Various methods are employed to inhibit, or suppress, the immune response:

- Generalized immunosuppression; glucocorticoids (inhibition of inflammatory cells), cytostatic drugs (endoxan, DNA alkylating agents, methotrexate, antimetabolites), and more specific immunosuppressants, e.g., cyclosporine A, FK506, rapamycin (inhibition of signal transduction in T cells, see Fig. 2.**11**, p. 73).

- Immunosuppression by antibodies, soluble cytokine receptors, deletion of T cells or T-cell sub-populations (anti-CD4, anti-CD8, anti-CD3, anti-Thy1, etc.). Administration of monoclonal antibodies directed against adhesion molecules and accessory molecules or cytokines and cytokine receptors. Administration of soluble cytokine receptors, or soluble CTLA4, in order to block B7-1 and B7-2 (important costimulators, see p. 71ff.).

- Specific tolerance induction or "negative immunization." Massive and depletive T-cell activation brought about by systemic administration of large amounts of peptides, proteins (risk of immunopathology), or cells (chimerism).

- Complete neutralization and elimination of the antigen with the purpose of preventing induction of an antibody response. Example; rhesus prophylaxis with hyperimmune serum.

Adaptive Immunotherapy

This involves in-vitro antigen stimulation, and consequent proliferation, of patient T-cell effector clones or populations ($CD8^+$ T cells or less specific **lymphokine-activated killer cells, LAK cells**), followed by transfusion of these cells back into the patient. This method is sometimes used as a means of limiting cytomegaly or Epstein-Barr virus infection of bone marrow recipients. The LAK cells also include less specific NK-like cells, which can be expanded with IL-2 in the absence of antigen stimulation.

Toxic antibodies are monoclonal antibodies to which toxins have been coupled. These are used as specific toxin transporters, administered directly, or with liposomes bearing anchored antibodies and containing a toxin or cytostatic drug.

Immunological Test Methods

Antigen and Antibody Assays

Immunoprecipitation in Liquids and Gels

Immunoprecipitate. Maximum precipitation results when both reaction partners are present in an approximately *equivalent ratio* (Fig. 2.**20**). In antibody excess, or antigen excess, the amount of precipitate is considerably reduced.

Double diffusion according to Ouchterlony. This technique allows for a *qualitative* evaluation of whether certain antibodies or antigens are present or not, plus determination of the degree of relationship between antibodies and antigens. It also provides information on whether different antigenic de-

Immunoprecipitation

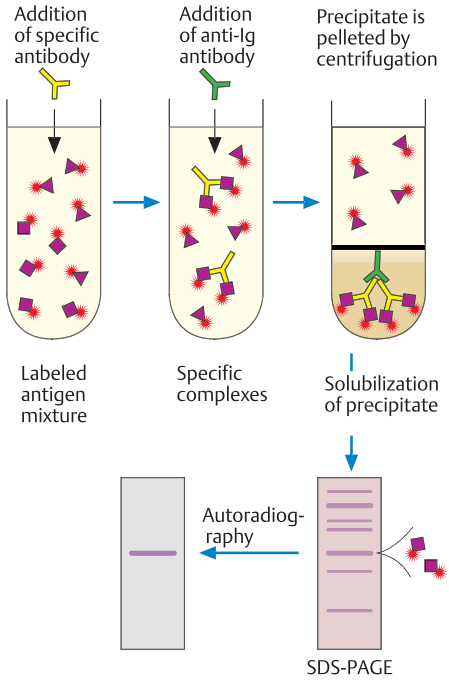


Fig. 2.**20** To identify the unknown antigen, a known specific antibody is added to an antigen mixture which is radio-, or otherwise-, labeled. The immune complexes are precipitated with the help of co-precipitating reagents (e.g., anti-immunoglobulin antibodies). The precipitate is thoroughly washed to remove unbound antigen, then dispersed into solution once again (e.g., in SDS), after which the components are separated using SDS polyacrylamide gel electrophoresis (SDS-PAGE). The labeled antigen is then rendered visible by means of autoradiography.

terminants are localized on the same, or on different, antigens; or whether different antibodies can bind to the same antigen (Fig. 2.**21**).

Radial immunodiffusion according to Mancini. This is a *quantitative* antigen assay based on a predetermined standard curve (Fig. 2.**22**).

Nephelometry. This method measures the amount of light scatter as a quantification of precipitation turbidity.

Immunoprecipitation Combined with Electrophoresis. Antigens are separated in an agarose gel by applying an electric current. The antibodies react by migrating in the gel, either without an electric field, or simultaneously within the electric field; and either in the same dimension as the antigens or in a second vertical step ("rocket" electrophoresis).

Immunoelectrophoresis according to Grabar and Williams. In the first instance serum proteins are electrophoretically separated within a thin agarose gel layer. A trough is then cut into the agar, next to the separated sample and parallel to the direction of migration along the entire migration distance, and anti-serum is applied to the trough. The antibodies diffuse into the gel, and precipitation lines are formed wherever they encounter their antigens. The

Double Diffusion According to Ouchterlony

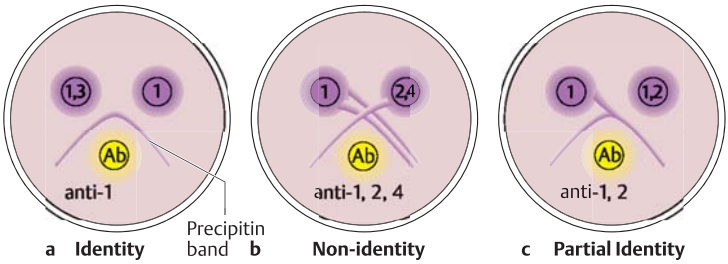


Fig. 2.**21** This technique facilitates assignment of antigens (violet) to a certain test antibody (yellow), or vice versa. The antigens and antibodies are pipetted into troughs within the gel and diffuse through this medium (the numbers designate the epitopes present). Where they meet lines of precipitation (known as precipitin bands) develop, indicating immune complex formation. **a** The antibodies precipitate identical epitopes (epitope 1) of both antigens, resulting in formation of precipitin bands which flow together to form an arch, mutually inhibiting their migration. In **b**, three independent precipitin bands form, indicating that the antibodies differentiate three different epitopes on three different antigens. **c** Epitope 1 of both antigen samples forms precipitin bands which flow together. Anti-2 migrates beyond the line of confluence into the area in which it precipitates with free antigen 1, 2 and forms a spur.

Radial Immunodiffusion According to Mancini

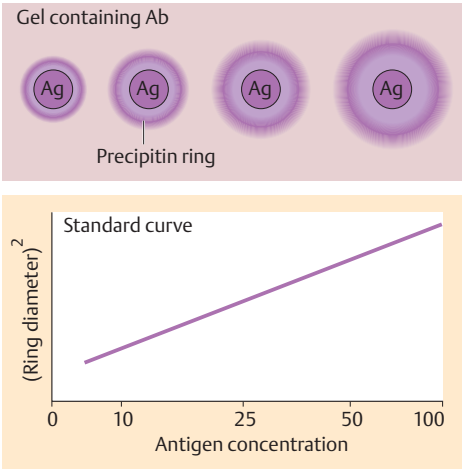


Fig. 2.**22** Quantitative assay of an antigen using a monospecific anti-serum which is mixed with agar and poured into a plate. The antigen is then diluted to different concentrations, and pipetted into wells that have been previously punched into the plate. Antigen-antibody complexes precipitate in the form of a ring around the well, the diameter of which is proportional to the antigen concentration. The result is a standard curve from which unknown test antigens can be quantified. Analogously, antibodies can also be quantified by mixing antigens into the gel.

precipitate can then be stained and evaluated. This older method is still used to identify paraproteins, monoclonal immunoglobulins, etc. (Fig. 2.**23**).

Electrophoresis plus antibody reaction: Western blotting. This method involves electrophoresis of proteins in a gel, coupled with detection by specific antibodies. The separated proteins are transferred to nitrocellulose, where they are identified with the help of specific antibodies (Fig. 2.**24**). Polyclonal sera is normally used for this purpose as monoclonal antibodies only rarely bind to denaturated and separated proteins.

Agglutination Reaction

Antibodies can agglutinate antigen-loaded particles (Fig. 2.**25**), whilst antigens can agglutinate antibody-loaded particles. Application: agglutination of bacteria or erythrocytes (e.g., blood group tests).

Immunoelectrophoresis According to Grabar and Williams

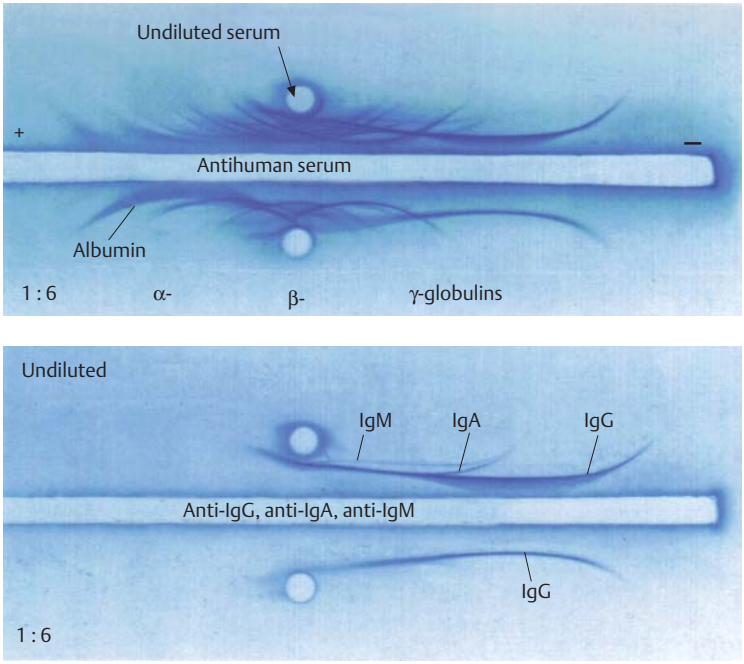


Fig. 2.**23** Serum is separated within agarose by an electric field, and rendered visible with anti-serum directed against human serum (above), or with selected specific antibodies (below).

Indirect hemagglutination. An antigen is fixed on the surface of erythrocytes and the antigen-loaded erythrocytes are then agglutinated using specific antibodies.

Hemagglutination inhibition test. The ability of a sample containing antigen to inhibit hemagglutination between antigen-loaded erythrocytes and antiserum is measured. This test is frequently used to quantify antibodies against hemagglutinating viruses (mainly influenza and parainfluenza viruses).

Antiglobulin tests according to Coombs. The direct Coombs test determines antibody binding directly to erythrocytes (e.g., anti-Rh antibodies agglutinate Rh^+ erythrocytes of neonates). The indirect Coombs test is suitable for detection of antibodies that have already bound to the Rh^+ erythrocytes of newborns (second pregnancy or sensitized mother), or which have been in-

Western Blotting

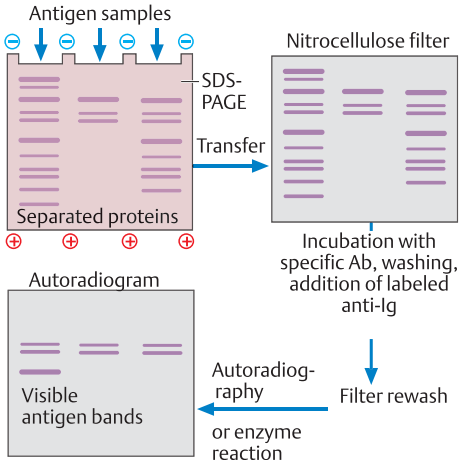


Fig. 2.**24** Antigen samples separated in a gel are transferred to nitrocellulose. Non-specific binding of the antibodies to the filter is then prevented with serum albumin or irrelevant proteins that do not cross-react with any of the antibodies used. Antibodies specific for the antigens being sought are then added. Once immune complexes have formed, the unbound antibodies are thoroughly washed away and the remaining bound antibodies are labeled using anti-immunoglobulin antibodies. These are in turn rendered visible by the autoradiographic procedure.

cubated in vitro with erythrocytes or antigenic particles. In all cases agglutination is detected using anti-Ig antibodies. Antigens can also be adsorbed to latex.

Complement Fixation Test (CFT)

CFT was formerly used to measure complement consumption by preformed antigen-antibody complexes. The unused complement is then detected by addition of a known amount of antibody-loaded erythrocytes. Should all of the erythrocytes be lysed, this indicates that no complement had been consumed and the CFT is negative. This method is no longer used very frequently, with the newer immunosorbent tests being preferred (RIA, ELISA, RAST, see below).

Direct and Indirect Immunofluorescence

Direct immunofluorescence. Immunofluorescence can be used for in-vivo detection of antibodies, complement, viruses, fungi, bacteria, or other im-

Hemagglutination

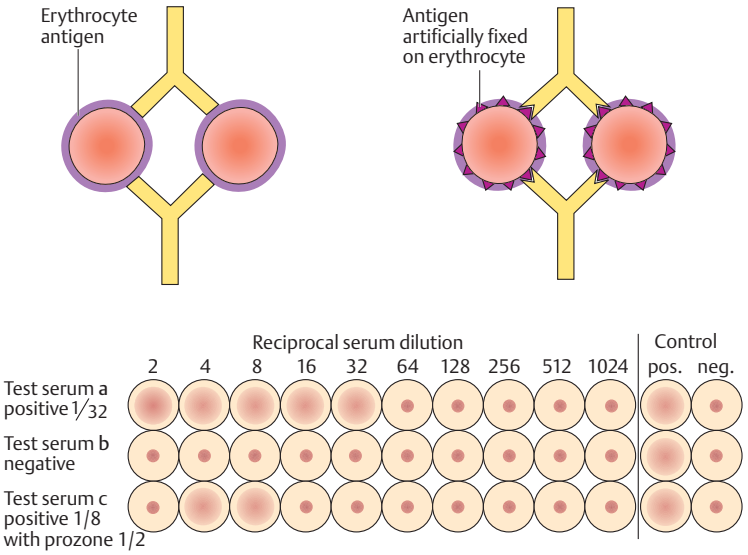


Fig. 2.**25** The hemagglutination test is based on the principle that erythrocytes cross-linked by antibodies settle to the bottom of the microtiter plate wells in mat-like aggregates (test sera **a** and **c**), whereas non-agglutinated erythrocytes collect at the lowest point of the wells to form a single "button" in the middle (test serum **b**). The test sera are first pipetted into the wells at the indicated dilutions, then the erythrocyte suspension is added. Non-specific agglutination is prevented by addition of an irrelevant protein. The test can be carried out using erythrocyte antigens (above left). Alternatively, other antigens can be fixed to the erythrocyte surface and the agglutination monitored (above right). The so-called "prozone" phenomenon results from non-specific blocking mechanisms present in sera which has not been sufficiently diluted.

mune factors present within patient cells and tissues. For this purpose tissue sections, or cell preparations, are treated with specific antibodies (anti-sera) which have been labeled with a fluorochrome (Fig. 2.**26a**). Antigen-antibody reactions can thus be detected using a fluorescence microscope. The fluorochrome absorbs light of a certain wavelength (e.g., UV light), and emits the light energy in the form of light at a different (visible) wavelength. The fluorochrome fluorescein isothiocyanate (FITC), which absorbs UV light and emits it as green light, is used most frequently (caution: bleaches out quickly!).

Antigen Detection Methods

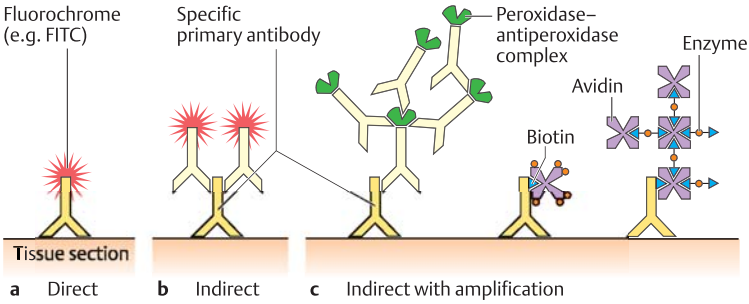


Fig. 2.**26** Immunofluorescence (**a**, **b**) is particularly suitable for the detection of antigens, or specific antibodies, fixed on plastic (solid phase) (ELISA) or present within a tissue section (immunohistology). For direct immunofluorescence (**a**) the specific primary antibody is labeled with a fluorochrome, or an enzyme (ELISA = enzyme-linked immunosorbent assay). The term indirect immunofluorescence is used when it is not the primary antibody being detected, but a secondary antibody which is directed against the unlabeled primary antibody and has also been labeled with a fluorochrome or enzyme (**b**). In most cases, this method achieves a certain degree of amplification. However, an even higher level of amplification can be achieved using preformed complexes of secondary antibody and enzyme (**c**). For the peroxidase method the detector enzyme is bound directly to the secondary antibody (peroxidase catalyzes a color reaction). In the biotin-avidin method the detector enzymes are coupled to either biotin or avidin.

Indirect immunofluorescence and enzyme histology. In this technique the specific or "first" antibody can be unlabeled. The antigen–antibody complexes that form are then detected using a labeled or "second" antibody, directed against the first antibody (Fig. 2.**26b**). Instead of fluorochromes, enzyme-labeled antibodies are now frequently used for tissue sections. The enzyme catalyzes the formation of a color signal following addition of a previously colorless detector substance. This color precipitate allows the direct observation of signals using a light microscopic, and exhibits little bleaching.

Indirect immunofluorescence can be used for the qualitative and quantitative analysis of antibodies directed against particular microbial antigens, or self-tissue antigens, within a patients serum. In the quantitative test, the antigen is fixed in a well or to a tissue section on a slide. The patient sample is repeatedly diluted by a factor of two and added to the antigen or section then rendered visible with a labeled anti–antibody.

There are two main methods of amplifying the immunohistological color signal:

The direct 'primary' antibody, or the detected 'secondary' antibody, is labeled with peroxidase. Following the antigen-antibody reaction, large preformed peroxidase-antiperoxidase complexes are added to the tissue section; these complexes can attach to the peroxidase-labeled antibodies, which are already specifically bound, thus amplifying the signal considerably (Fig. 2.**26c**).

Similarly, biotinylated antibodies can be used. The vitamin biotin is bound with strong affinity by avidin, a basic glycoprotein. Various colorants or enzymes coupled to avidin thus facilitate the color reactions. Such reactions can be amplified on the tissue section by adding preformed biotin-avidin-peroxidase complexes that bind to those biotin-coupled antibodies which have already been bound.

Radioimmunological and Enzyme Immunological Tests

Radioimmunoassay (RIA) and enzyme immunoassay (EIA), also known as ELISA (enzyme-linked immunosorbent assay) (Fig. 2.**28**), are now used very frequently to test for antigens and antibodies. All absorbency tests involve the fixation of antigens or antibodies to a plastic surface. The lower detection limit is a few nanograms. This method forms the basis of modern hepatitis serology, HIV tests, and tests for autoantibodies, lymphokines, cytokines, etc. All of these assays can be performed in a direct form (different sandwich combinations of antigen, antibody and anti-antibody, Fig. 2.**27**)

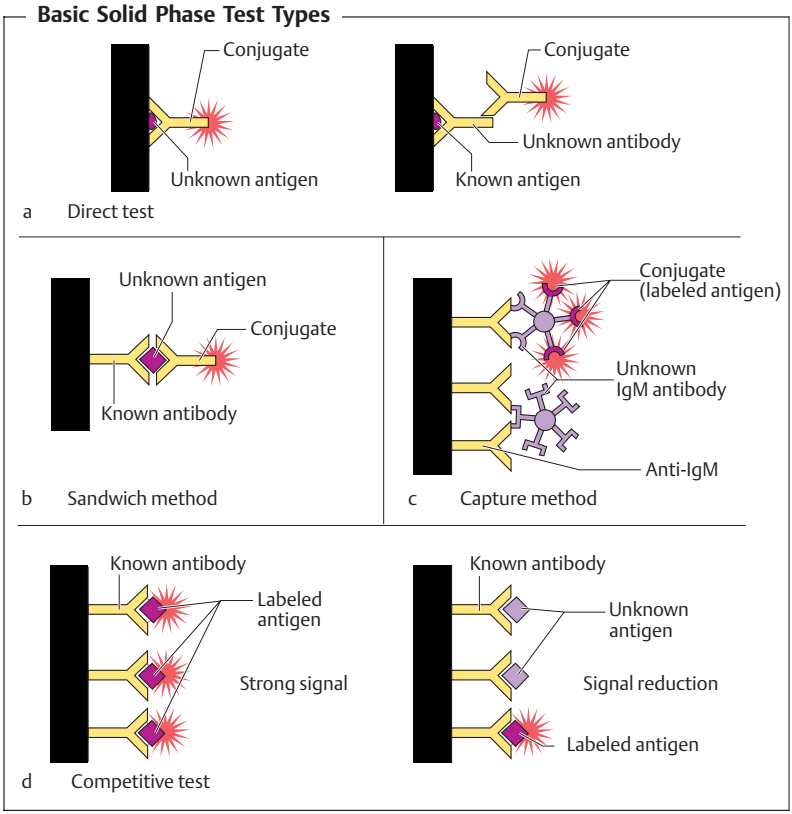
Fig. 2.**27** For solid phase tests both the antigen and antibody are bound to a solid phase (e.g., plastic surface). Various methods are then used to detect any interaction between the antigen and antibody. In the direct test (**a**) an immobilized, unknown, antigen can be detected using a fluorescent-labeled antibody. If the immobilized antigen is known, this test method can also be used to detect an antibody bound to the antigen. In the sandwich method (**b**) a known antibody is immobilized. Detection of antibody-antigen binding is then performed using a second, labeled antibody which interacts with the antigen at a different site. The capture method (**c**) can be used to detect any antigen, for instance IgM antibodies. First, anti-IgM antibodies are immobilized, then serum containing IgM is added to them. The bound IgM can then bind a foreign antigen (e.g., a virus). The detection procedure next makes use of either the labeled foreign antigen or a specific, additionally labeled, antibody which binds to the bound antigen but not to the plastic bound antibody. In the competition or competitive inhibition test (**d**) antibodies are immobilized, and labeled antigens are then bound to them. An unlabeled (unknown) antigen is added, which competes with the labeled antigen. The level of interaction between the antibody and the unknown antigen is then determined by measuring attenuation of the signal. ▶

or as competition assays. Fig. 2.**28** illustrates the quantitative IgE assay, Fig. 2.**29** the procedure for detection of specific IgE in patient sera. Analogous procedures are used to detect specific antibody-binding cells or cytokine-releasing T cells (Fig. 2.**30**).

In-Vitro Cellular Immunity Reactions

Isolation of Lymphocytes

The methods used to measure cellular immunity are experimentally complex. The first step is to isolate human lymphocytes from blood, which can be achieved using Ficoll density gradient centrifugation. Certain lymphocyte



Radioimmunosorbent Test (RIST)

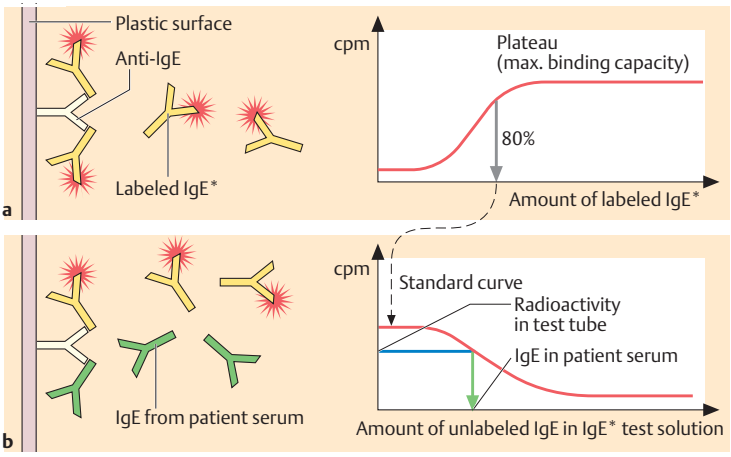


Fig. 2.**28** RIST is a competitive radioimmunoassay (RIA) used for quantitative measurement of antibodies of any given Ig class (in this example total IgE) within patient serum. Anti-IgE antibodies are allowed to adsorb to the solid phase (plastic surface). In the first instance, defined concentrations of radiolabeled IgE (IgE*) are used to determine the maximum binding capacity of these antibodies (**a**). The actual test (**b**) is then performed using the IgE* concentration determined to result in 80% saturation of the fixed antibodies: The IgE* test solution is added to the fixed anti-IgE antibodies and the patient serum is then added by pipette. The more IgE the serum contains, the more IgE* will be displaced by the patients antibodies, and the lower the radioactivity level will be in the test tube. The IgE concentration in the patient serum is then calculated based on a standard curve established previously by progressively "diluting" the IgE* test solution with unlabeled IgE.

populations can be coated with magnetic beads, or sheep erythrocytes loaded with specific antibodies, then purified using a magnet or a Ficoll gradient. The fluorescence-activated cell sorter (FACS, Fig. 2.**31**, p. 133) is now regularly used for this purpose. In this assay, monoclonal antibodies labeled with various fluorochromes directed against cell surface antigens (such as CD4, CD8), or against intracellular cytokines (which involves the use of detergents to increase the permeability of the cell membrane), are incubated with the isolated blood lymphocytes. Alternatively, antigen-specific T lymphocytes can be labeled with MHC class I or II plus peptide tetramers (see below). Following incubation, and several washing steps, the equipment identifies and counts the antibody-loaded lymphocytes, employing magnetic pulse sorting as required.

Radioallergosorbent Test (RAST)

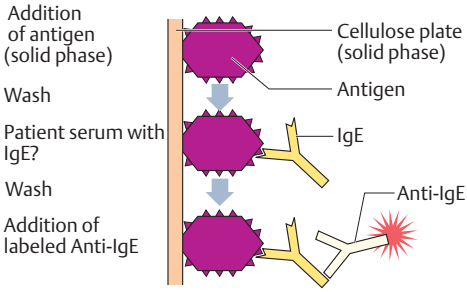


Fig. 2.**29** This test is a highly sensitive detection method for the presence of specific IgE in patient serum. Antigen is bound covalently to a cellulose plate (solid phase). Any IgE in the serum that binds to the antigen is then detected using radiolabeled anti-IgE antibodies.

Tetramer test for detection of specific T cells (Fig. 2.**32**, p. 134): recombinant MHC class I antigen coupled to biotin, labeled with avidin, and correctly folded together with peptide and β_2 microglobulin, forms tetramers; which are recognized by specific TCRs. Subsequent analysis of tetramer binding using FACS equipment is based on the color indicator of the avidin (fluorescein, phycoerythrin, etc.). Tetramers specific for MHC class II antigens plus

ELISPOT Assay

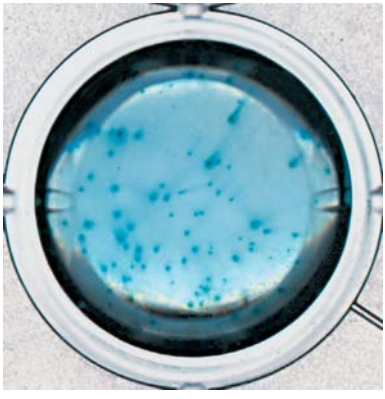


Fig. 2.**30** In the ELISPOT assay antigens, or specific anti-IL antibodies, are applied to the plastic surface. It is then possible to determine the number of immune cells releasing antibodies specific for the applied antigen, or releasing interleukins that are recognized by the applied anti-IL antibodies. Following incubation at 37 °C, the immune complexes which form around these cells can be visualized using a covering agarose layer which includes an enzyme-coupled antibody. These enzymes catalyze a color reaction, resulting in the formation of color spots, each of which will correspond to a single cell producing the specific antibody or interleukin.

peptide can theoretically be used to assay specific $CD4^+$ T cells, but are still difficult to manufacture. Using the tetramer test, specific T cells can be detected directly from blood or lymphoid organs. Histological applications are feasible, but still difficult.

Lymphocyte Function Tests

Certain functions of isolated lymphocyte populations can be determined by a number of methods:

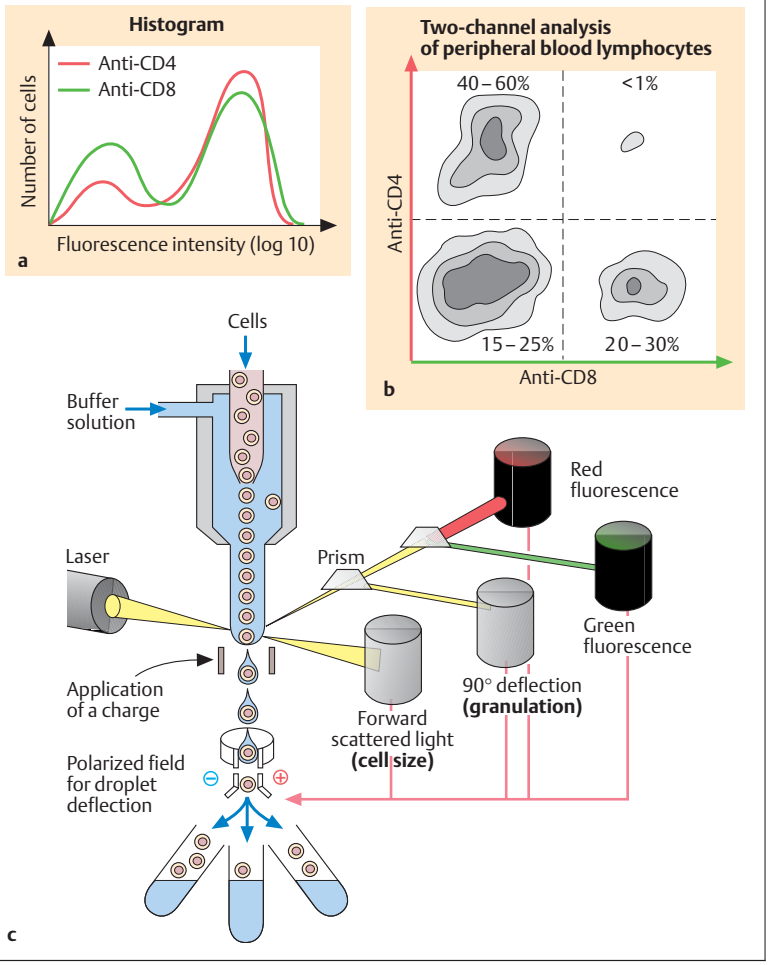
- Determination of the *number of cells producing antibodies*, e.g., the hemolytic plaque assay in which antibody production is tested by adding antigen-coupled erythrocytes. In the vicinity of antibody-secreting cells, the erythrocytes are covered with antibodies and can be lysed by addition of complement. Today, ELISA methods are more often used than erythrocytes (ELISPOT).
- *ELISPOT ASSAY*: used to measure antibody-producing, or IL-releasing, lymphocytes. The antigen or anti-IL antibody is fixed on a plastic surface. Lymphocytes are then placed over this, within a thin layer of agar medium. When the cells are incubated at 37 °C, they may secrete the antibodies or IL recognized by the corresponding test substances. After a certain period of time, the cell layer is shaken off and the preparation is thoroughly washed. The bound material can then be developed using an overlaid semisolid agar, as for the ELISA method. The enzyme reaction generates spots of color, each of which corresponds to a cell, and which can be counted (Fig. 2.**30**).
- Measurement of the *release capacity of cytokines*, or detection of mRNA, is also possible with the ELISPOT assay.
- *Lymphocyte stimulation assay*: isolated lymphocytes are incubated with antigen in culture medium. Measuring the ^{3}H-thymidine incorporation,

Fig. 2.**31** This device analyzes cells by means of fluorescent-labeled antibodies directed against cell surface antigens – or for permeabilized cells, directed against internal cell antigens. In the example shown, peripheral blood lymphocytes (PBL) are incubated with monoclonal antibodies specific for CD4 or CD8, resulting in the distribution of fluorescence intensity as indicated in **a**. In **b** the labeling of different cell populations with anti-CD4 or anti-CD8 is shown. By this means, the percentages of the subpopulations in the total population can be determined. The fluorescence-activated cell sorter shown in (**c**) makes use of this data. By means of vibration, the cell stream is broken up into fine droplets which, depending on the fluorescence and sorting settings used, are charged just before they are separated and ideally contain one cell each. Certain parameters are measured for each cell with the help of a laser beam, where-upon the droplets are deflected into the intended containers by the + and – plate fields. ▶

interleukin release, or a pH transition, can determine whether antigen-specific lymphocytes are present or whether polyclonal T-cell responses (concanavalin [ConA], phytohemagglutinin [PHA]) or B-cell responses (lipopolysaccharide [LPS], pokeweed mitogen [PMA]) were induced.

■ Mixed lymphocyte reactions are used to measure *alloreactivity* (proliferation, cytotoxicity), mainly between recipients and donors of organ or bone

Fluorescence-Activated Cell Sorter (FACS)



Tetramer Assay

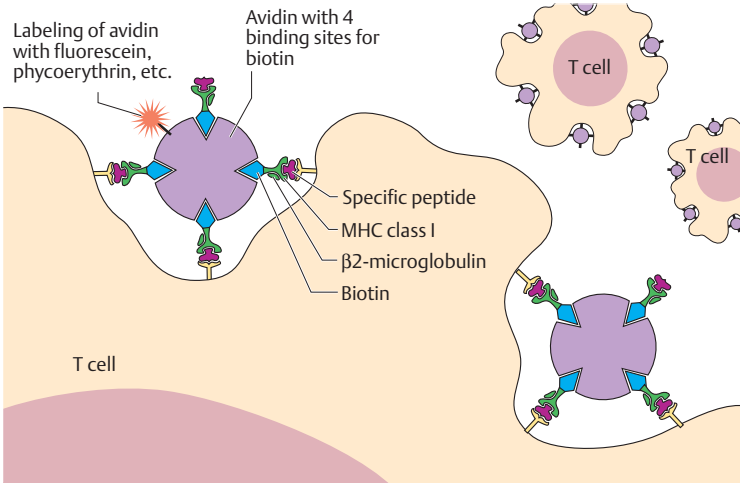


Fig. 2.**32** The tetramer assay is an antigen-specific binding test for living T cells. Complexes comprising biotin-coupled MHC class I heavy chains, β_2 microglobulin, and specific peptide are properly folded, washed, then bound to avidin (which contains four binding sites for biotin). The resulting tetrameric complexes are then incubated with a population of T cells. Those T cells expressing the appropriate T-cell receptor will bind to two or three of the exposed MHC class I-peptide complexes present on each tetramer. Labeling of the avidin component with fluorescein, phycoerythrin, or other fluorescent substances then permits FACS analysis of tertramer binding T cells.

marrow transplants. This test is based on the principle that T lymphocytes are stimulated to proliferate by nonself MHC class I or II antigens and to develop into cytotoxic T cells directed against class I.

- Chromium release assay measures *cytolytic activity*, mainly by $CD8^+$ T cells, directed against allogeneic, virus-infected, or peptide-loaded target cells. The target cells are incubated with ^{51}Cr which the cells incorporate. They are then cultivated with effector cells for 4–6 hours. When the target cells are lysed chromium is released into the culture medium, following which it can be quantitatively measured.

- Assay of intracellular cytokines. Following a brief stimulatory culture (six hours), the cells are rendered permeable using a mild detergent so that specifically labeled antibodies can diffuse into the cells. Labeled cells can then be analyzed by FACS equipment (or by a microscope).

Table 2.**13** Important CD Antigens

Designation (alternatives)	Cells which express the antigen	Functions
CD1	Cortical thymocytes, Langerhans cells, dendritic cells, B cells, intestinal epithelium	MHC class I-like molecule, associated with β_2 microglobulin. Possible special significance in specialized antigen presentation
CD2 (LFA-2)	T cells, thymocytes, natural killer cells	Adhesion molecule which binds to CD58 (LFA-3) and can activate T cells (LFA = lymphocyte function antigen)
CD3	Thymocytes, T cells	Associated with the antigen receptor of T cells, and is necessary for T-cell receptor surface expression and signal transduction
CD4	Several groups of thymocytes, T helper cells, and inflammatory T cells (about two-thirds of the peripheral T cells), monocytes,macrophages	Co-receptor for MHC class II molecules. Binds signal transducers via cytoplasmic portion. Receptor for gp120 in HIV-1 and HIV-2
CD5	Thymocytes, T cells, a subgroup of B cells	Binds to CD72
CD8	Several groups of thymocytes, cytotoxic T cells (about one-third of the peripheral T cells)	Co-receptor for MHC class I molecules, binds signal transducer via cytoplasmic portion
CD10	B and T precursor cells, bone marrow stroma cells	Zinc metal proteinase, and marker for acute lymphoid leukemia of pre-B cells
CD11a (α chain)	Lymphocytes, granulocytes, monocytes, and macrophages	α subunit of β_2 integrin LFA-1 (associated with CD18). Binds to CD54 (ICAM-1), CD102 (ICAM-2), and ICAM-3 (CD50)
CD19	B cells	Forms a complex with CD21 (CR2) and CD81 (TAPA-1), Co-receptor for B cells

Table 2.13 *Continued: Important CD Antigens*

Designation (alternatives)	Cells which express the antigen	Functions
CD21 (CR2)	Mature B cells, follicular dendritic cells	Receptor for the complement components (CR) C3d and the Epstein-Barr virus. Forms a co-receptor for B cells together with CD19 and CD81
CD22	Mature B cells	Adhesion of B cells to monocytes and T cells
CD23 (FcεRII)	Mature B cells, activated macrophages, eosinophils, follicular dendritic cells, blood platelets	Low-affinity receptor for IgE, and ligand for the CD19:CD21:CD81 coreceptor
CD25 (Tac)	Activated T cells, B cells, and monocytes	α chain of the IL-2 receptor, associated with CD122 and the IL-2Rγ chain
CD26	Activated B and T cells, macrophages	A protease which may be involved in HIV entry into host cells
CD28	Subgroup of T cells, activated B cells	Activation of naïve T cells. A receptor for costimulatory signal (signal 2); binds CD80 (B7.1) and B7.2
CD29	Leukocytes	β_1 subunit of γ_1 integrins, associated with CD49a in VLA-1 integrin
CD34	Hematopoietic precursor cells, capillary endothelium	Ligand for CD62L (L-selectin)
CD35 (CR1)	Erythrocytes, B cells, monocytes, neutrophils and eosinophils, follicular dendritic cells	Complement receptor 1; binds C3b and C4b. Also mediates phagocytosis
CD38	Early B and T cells, activated T cells, germinal center B cells, plasma cells	B-cell proliferation?
CD39	Activated B cells, activated natural killer cells, macrophages, dendritic cells	Unknown fuction, but may mediate adhesion of B cells

Table 2.13 *Continued: Important CD Antigens*

Designation (alternatives)	Cells which express the antigen	Functions
CD40	B cells, monocytes, dendritic cells	Receptor for the costimulatory signal for B cells; binds CD40 ligand (CD40-L)
CD40L	Activated CD4 T cells	Ligand for CD40
CD44 (Pgp-1)	Leukocytes, erythrocytes	Binds hyaluronic acid and mediates adhesion of leukocytes
CD45, RO, RA, RB (leukocyte common antigen, LCA), T200, B220	Leukocytes	A tyrosine phosphatase which enhances signal mediation via the antigen receptors of B and T cells; alternative splicing results in many isoforms (see below)
CD54	Hematopoietic and nonhematopoietic cells	Intercellular adhesion molecule (ICAM-1); binds the CD11a/CD18 integrin (LFA-1) and the CD11b/CD18 integrin (MAC-1); receptor for rhinoviruses
CD55 (DAF)	Hematopoietic and nonhematopoietic cells	Decay accelerating factor (DAF); binds C3b and cleaves C3/C5 convertase
CD62E (ELAM-1, E-selectin)	Endothelium	Endothelial leukocyte adhesion molecule (ELAM); binds sialyl-Lewis x and mediates rolling of neutrophilic cells along endothelium
CD64 (FcγRI)	Monocytes, macrophages	High-affinity receptor for IgG
CD80 (B7.1) CD86 (B7.2)	Subgroup of B cells	Costimulators which act as ligands for CD28 and CTLA-4
CD88	Polymorphonuclear leukocytes, macrophages, mast cells	Receptor for the complement component C5a
CD89	Monocytes, macrophages, granulocytes, neutrophils cells, subgroups of B and T cells	IgA receptor?
CD95 (APO-1, Fas)	Many different cell lines; unclear distribution in vivo	Binds TNF-like Fas ligands; induces apoptosis

Table 2.**13** *Continued: Important CD Antigens*

Designation (alternatives)	Cells which express the antigen	Functions
CD102 (ICAM-2) (intercellular cell adhesion molecule)	Resting lymphocytes, monocytes, endothelial cells (in which expression is most pronounced)	Binds CD11a/CD18 (LFA-1), but not CD11b/CD18 (MAC-1)
CD106 (VCAM-1) (vascular cell adhesion molecule)	Endothelial cells	Adhesion molecule; ligand for VLA-4 (very late antigen)
CD115	Monocytes, macrophages	Receptor for the macrophage colony-stimulating factor (M-CSF)
CD116	Monocytes, neutrophils, and eosinophils, endothelium	α chain of the receptor for the granulocyte-macrophage colony-stimulating factor (GM-CSF)
CD117	Hematopoietic precursor cells	Receptor for stem cell factor (SCF)
CD118	Widespread	Receptor for alpha/beta interferons (IFNα/β)
CD119	Macrophages, monocytes, B cells, endothelium	Receptor for gamma interferon (IFNγ)
CD120a	Hematopoietic and nonhematopoietic cells	Most pronounced on epithelial cells

Acknowledgment
The expert editing and translating help of Nicola Harris PhD is gratefully acknowledged.

Glossary

ABC: Antigen-binding cell.
ABS: Antigen-binding site on an antibody.
ADCC: Antibody-dependent cell-mediated cytotoxicity.
Adjuvant: A substance which intensifies the immune response against an antigen, in a immunologically non-specific manner.
AFC: Antibody-forming cell.
Affinity: A measure of binding strength between an antigen determinant (epitope) and the binding site of an antibody (paratope).
Affinity maturation: An increase in the average antibody affinity acquired during the course of a secondary and following immune responses.
AFP: α-fetoprotein.
Allele: Gene locus variations within a species.
Allergy: An altered response following secondary contact with the same antigen, also defined as type I hypersensitivity.
Allogeneic: Refers to the genetic variety contained within a species.
Allotransplant: Transplanted allogeneic tissue.
Allotype: Different forms of a protein product, usually Ig, recognized as an antigen by another individual of the same species.
Alternative pathway: Activation of the complement system via C3 or other factors, but not via C1q.
ANA (anti-nuclear antibodies)**:** Autoantibody directed against DNA contained within the cell nucleus.
Anaphylatoxins: Complement fragments (C3a and C5a), responsible for mediating mast cell degranulation.
Anaphylaxis: An antigen-specific, primarily systemic IgG- or IgE-mediated-immune response.
Antigens: Molecules which are usually characterized by complex folding, and which can be recognized by antibodies.
Antibody: A molecule which binds to a specific antigen.
APC: Antigen-presenting cell.
Atopic: Increased susceptibility to the clinical manifestations associated with type I hypersensitivity (e.g. eczema, asthma, and rhinitis).
Autologous: Derived from the same individual (or inbred strain).
Autosomes: All chromosomes other than the X or Y sex chromosomes.
Avidity: A measure of the functional binding strength between an antibody and its antigen; dependent on affinity and valences (number of binding sites).
BCA-1: B-cell attractant.
BCG: Bacillus Calmette-Guerin. An attenuated form of *Mycobacterium tuberculosis*.
BCGF: B-cell growth factor.
Bence-Jones proteins: Free light chains of Ig present in the serum and urine of multiple myeloma patients.
Bursa fabricii: Lymphoepithelial organ adjacent to the cloaca of birds, in which B cells mature.
C: Complement (C1–9).
C domain: Constant component of Ig.
C3b inactivator: A component of the complement system, known as factor I.
Capping: Aggregation of surface molecules on the cell membrane.
Carrier: The part of a molecule which is recognized by T cells during an immune response.
CBR: Complement-binding reaction.
CCR: A receptor for those chemokines which contain adjacent cysteine-cysteines (CXC or CXXC, cysteines separated by one or two amino acids).
CD marker: Cluster determinant or cluster of differentiation, characteristic of distinct lymphocyte subpopulations.
CDR: Complementarity determining regions (hypervariable antibody regions).
Chemokines: Chemoattractant cytokines

Chimera: A single host bearing cells derived from genetically distinct individuals.

CLIP: A protein which blocks the binding groove of MHC class II prior to its inclusion in the phagolysosome (class II-inhibiting protein).

CMI: Cell-mediated immunity.

Cobra venom factor: A component of cobra venom which exhibits enzymatic activity corresponding to the activity of mammalian C3b.

Combining site: The configuration on an antibody which forms a link with antigen determinants (ABS).

Complement system: A group of serum proteins that are activated in cascades; usually via antibodies, but in some cases directly by infectious agents. Plays an important role during inflammation, chemotaxis, cytolysis, and phagocytosis.

Con A (concanavalin A): A T-cell mitogen.

Cryoglobulin: Antibodies in immune complexes that can be precipitated at 4 °C.

CSF: Colony-stimulating factor (also: cerebrospinal fluid).

CTL: Cytotoxic $CD8^+$ T cell.

CXCR: Receptor for those chemokines which contain a cysteine-x-cysteine motif.

Cyclophosphamide: A toxic substance frequently used to induce immunosuppression.

Cyclosporine A: An immunosuppressant used for the prevention of rejection reactions.

Cytophilic: Exhibiting an affinity towards cells (i.e., binds to cells).

Cytostatic: Exhibiting an inhibitory effect on cell proliferation.

Cytotoxic: Exhibiting a destructive effect towards target cells.

DARC: Duffy antigen receptor for chemokines.

Dendritic cells: Professional APCs derived from the bone marrow. Dendritic cells are mobile and function to transport antigen into lymphoid organs. In the skin they are known as *Langerhans* cells, on the way to the lymph nodes *veiled* cells, and in the lymph nodes *interdigitating* cells.

Desensitization: Repeated exposure to small amounts of an antigen, against which the host shows an allergic reaction; the aim being to downregulate IgE production and upregulate IgG production.

DiGeorge syndrome: Congenital thymic hypoplasia.

DNP: Dinitrophenol, a frequently used small hapten.

Domain: A peptide region with a stable tertiary structure. Immunoglobulins (Ig), MHC class I, and MHC class II molecules all contain comparable Ig domains.

DTH: Delayed type hypersensitivity; A delayed cellular type IV response.

EAE: Experimental allergic encephalitis.

ELISA: Enzyme-linked immunosorbent assay.

ELISPOT: A modified ELISA method used for the detection of specific cell secretion products.

Endotoxins: Bacterial toxins; largely comprised by lipopolysaccharides (LPS) from Gram-negative bacteria.

Eotaxin: A chemokine which regulates eosinophil migration.

Epitope: A special region within an antigen, which is recognized by an antibody binding site.

Epstein-Barr virus: A herpes virus capable of transforming human B cells, and for which B cells possess a special receptor (EBVR). The causative agent of infectious mononucleosis (Pfeiffer disease).

Exon: A protein-coding gene fragment.

Fab: The part of the antibody molecule which contains the antigen-binding site following treatment with papain; comprises a light chain and the first two domains of the heavy chain.

FACS: Fluorescence-activated cell sorter.

Fc: Antibodies use the Fc fragment to bind to cellular receptors (FcR) and C1q complement components.

FcR: Fc receptor.

Fractalkine: A chemokine expressed by endothelial cells; has effects on inflammation and other processe.

Freund's adjuvant (FA): A water-in-oil emulsion. Complete FA contains killed *Mycobacterium tuberculosis*, whilst incomplete FA does not.

GALT: Gut-associated lymphoid tissue.

Gammaglobulins: The serum fraction which migrates most rapidly towards the anode during electrophoresis. Contains all five classes of immunoglobulins.

Gel diffusion test: Immunoprecipitation test; Antigens and antibodies diffuse towards one another, forming a stainable precipitate at the equivalence zone (Ouchterlony test).

Germ line: The genetic material of gametes. Mutations in the germ line, unlike somatic mutations, are inherited by progeny.

GVH: Graft-versus-host reaction; rejection of host tissue by transplanted cells.

H-2: Main histocompatibility complex of mice.

Haplotype: The set of genetic determinants present on one chromosome or chromosome set.

Hapten: A small molecule which can function as an epitope by itself, without being coupled to a carrier, but which alone does not elicit an antibody response.

Helper cells: The $CD4^+$ subclass of T cells which are functionally important for B cells, and which release cytokines; a single helper T-cell clone is specific for one peptide presented by a specific MHC class II molecule.

Hereditary angioedema: Result of congenital C1 inhibitor deficiency.

Heterologous: Belonging to another species.

HEV: High endothelial venules; these are specialized to allow the movement of lymphocytes from the blood into the lymph nodes.

High responder: Individuals (or inbred strains) which exhibit a strong immune response against a defined antigen.

Hinge region: The segment of an immunoglobulin heavy chain which lies between the Fc and Fab regions.

Histocompatibility: Quality which determines rules of acceptance or rejection of a transplant.

HLA: Human leukocyte antigen coded for by the human major histocompatibility gene complex (MHC).

Homologous: Belonging to the same species.

Humoral: Any factor present within extracellular body fluids (e.g. serum, lymph).

HVG: Host-versus-graft reaction. Rejection of transplanted cells by host tissue.

Hybridoma: An antigen-specific B cell that has been successfully fused with a myeloma cell.

Hypervariable region: The three most variable segments present within the V domains of immunoglobulins and T-cell receptors.

Idiotype: The antigenic characteristic of the ABS region of an antibody.

IFN: Interferons; cellular derived substances which contribute to nonspecific cellular resistance, particularly with regard to viral infections.

IL: Interleukins; short-lived substances which mediate the transfer of information between distinct cells (both of the immune system and other tissues).

Immune complex: The product of an antigen-antibody reaction; may also contain components of the complement system.

Immune paralysis: Temporary inability to produce a specific immune response usually resulting from the presence of excessive antigen.

Immunity: Actively or passively acquired immune protection against pathogens and other antigens.

Immunoconglutinins: Auto-antibodies directed against complement components.

Immunofluorescence: Rendering certain antigens visible by binding of a specific fluorescence-labeled antibody.

Immunogen: Any substance which can elicit a specific immune response.

Immunological memory: The ability to produce a faster and stronger immune response following a second, or subsequent, encounter with the same antigen.

Intron: The gene segment present between two exons.

Ir genes: Immune response genes; an early designation used for MHC genes. These code for MHC molecules which control peptide presentation, and thus directly determine the specificity and strength of an immune response.

Isologous: Of identical genetic constitution.

Isotype: The "isotypic" variants of certain proteins coded within the genome, which are identical for all individuals of a species (e.g., immunoglobulin classes).

J genes: Joining genes; a set of gene segments contained within the genetic loci of the heavy and light immunoglobulin chains, or T-cell receptor chains.

K cells: Killer cells; a group of lymphocytes bearing Fc receptors which can destroy their target cells by means of antibody-dependent cell-mediated cytotoxicity (ADCC).

Kupffer cells: Phagocytic cells present in the hepatic sinusoids.

LAK: Lymphokine-activated killer cells (lymphocytes).

LARC: Liver and activation-regulated chemokine.

LCM: Lymphocytic choriomeningitis; an non-bacterial, viral, meningitis.

LGL: Large granular lymphocyte.

Low responder: Individuals (or inbred strains) which exhibit a weak immune response against a given antigen.

LPS: Lipopolysaccharide; a component of the cell wall of certain Gram-negative bacteria, which acts as a B-cell mitogen.

MALT: Mucosa-associated lymphoid tissue.

MBP: Myelin basic protein; functions as an antigen in experimental allergic encephalitis (and probably in multiple sclerosis).

MCP: Monocyte chemoattractant protein.

MDC: Macrophage-derived chemokine.

MHC: Major histocompatibility complex; the main genetic complex responsible for determining histocompatibility. This gene complex codes for the most important transplantation antigens (HLA antigens) in humans. MHC class I molecules are associated with β_2 microglobulin, class II molecules consist of two noncovalently bound transmembrane molecules. The actual function of MHC I, and MHC II, molecules is to present antigenic peptides on the cell surface. Class III molecules comprise complement components, cytokines, and so on.

MHC restriction: Resulting from the interaction of T lymphocytes with other cells, and being controlled by recognition of MHC-presented peptides by the TCR.

MIF: Migration inhibition factors; a group of peptides produced by lymphocytes which inhibit macrophage migration.

MIG: A monokine induced by interferon gamma.

β_2 microglobulin: A protein component of MHC class I molecules.

MIP: Macrophage inflammatory protein.

Mitogen: Any substance which can alone stimulate cells, particularly lymphocytes, to undergo cell division.

MLC: Mixed lymphocyte culture. An in-vitro assay which measures the stimulation response of lymphocytes as alloreactive cytotoxic T-cell reactivity.

MLR: Mixed lymphocyte reaction. An in-vitro assay which measures the stimulation response of lymphocytes as alloreactive proliferation (determined by ^{3}H-thymidine incorporation).

Monoclonal: Any substance derived from a single cell clone, for example monoclonal antibodies.

Myeloma: A B-cell lymphoma, which produces antibodies (plasmocytoma).

NK cells: Natural killer cells. Non-MHC-restricted lymphocytes capable of recognizing and destroying certain cells

that are either virally infected or tumorous.

Nude mice: A mouse strain which carries a spontaneous mutation resulting in the animals having no hair, and usually being athymic.

NZB/W: A strain of mouse bred as an animal model for systemic lupus erythematosus.

Opsonization: Depositions of proteins on an infectious pathogen, that facilitate phagocytosis of the pathogen (e.g., antibodies and C3b).

Paratope: The part of an antibody molecule which contacts the antigenic determinant (epitope); the antigen-binding site (ABS) on the antibody.

PC: Phosphorylcholine; a commonly used hapten found on the surface of a number of microorganisms.

PCA: Passive cutaneous anaphylaxis; a classic detection reaction for antigen-specific IgE.

PFC: Plaque forming cell; an antibody-producing cell which can be detected by the hemolysis plaque test.

PHA: Phytohemagglutinin; a mitogen for T cells.

Plasma cell: An antibody-producing B cell which has reached the end of its differentiation pathway.

PMN: Polymorphonuclear neutrophilic granulocytes.

Pokeweed mitogen: A mitogen for B cells.

Polyclonal: A term describing products derived from a number of different cell clones (e.g., polyclonal antibodies).

Primary lymphoid tissues: Thymus, bursa of Fabricius (in birds), bone marrow.

Primary response: The immune response which follows an initial encounter with a particular antigen (see priming, secondary response).

Priming: Following an initial contact with an antigen, an immunocompetent cell becomes sensitized or "primed."

Prozone phenomenon: Lack of a measurable response at high-test substance concentrations.

Pseudoalleles: Tandem variants of a gene, which do not occupy a homologous position on the chromosome.

Pseudogenes: Genes containing structures which are homologous to other genes, but which cannot be expressed.

RANTES: Regulated on activation; this is normally expressed and secreted by T cells.

Reagin: Historical term for IgE.

Rearrangement: For instance the rearrangement of genetic information in somatic B and T cells.

Recombination: A process by which genetic information is rearranged during meiosis.

Reticuloendothelial system (RES): Phagocytic cells distributed within the supportive connective tissue of the liver, spleen, lymph nodes, and other organs (e.g. sinus endothelial cells, Kupffer cells, histiocytes).

Rhesus (Rh) antigens: Antigenic proteins present on the surface of erythrocytes in approximately 85% of all humans.

Rheumatoid factor (RF): Autoantibodies—these are usually IgM but can also be of the IgG and IgA isotypes—which are specifically directed against the body's own IgG molecules.

SCID: Severe combined immunodeficiency disease; a congenital deficiency of the humoral and cellular immune system, resulting from a lack of both T and B cells. The animal model is the SCID mouse and is a spontaneous mutant.

SDF-1alpha: Stromal cell-derived factor.

Secretory piece: An IgA-associated polypeptide produced by epithelial cells, and which facilitates the transmembrane transport of IgA.

Secondary response: The immune response which follows a second encounter with a specific antigen.

Serum sickness: An inflammatory type III reaction, occurring after repeated injection of a foreign protein.

SLC: Secondary lymphoid organ chemokine.

SLE: Systemic lupus erythematosus.

Somatic mutation/recombination: Rearrangements of genes in somatic cells (as opposed to germ line cells), resulting in a newly combined DNA sequence which is not heritable.

Splenomegaly: Splenic enlargement; often observed in cases of hematopoietic cell tumors, vascular circulatory problems, or following various parasitic infections. Can also be used as a measure of GVH reactions.

SRBC: Sheep red blood cells (erythrocytes).

Stripping: The process by which antibodies remove antigen determinants from target cells.

Suppressor cell: A proposed antigen-specific T-cell subpopulation which acts to reduce the immune responses of other T cells or B cells. This suppression can also be of a nonspecific nature.

Syngeneic: Animals produced by repeated inbreeding, or monozygotic twins, which are considered syngeneic when each pair of autosomes within the individuals is identical.

TATA: Tumor-associated transplantation antigens.

Tc: Cytotoxic T cell ($CD8^+$ T cell) or CTL.

TCGF: T-cell growth factor; identical with interleukin 2 (IL-2).

T-dep/T-ind: T cell-dependent/T cell-independent; an antibody response to T-dependent antigens is only possible if (MHC-restricted) T-cell help is also available.

T-DTH: A T cell that contributes to delayed type hypersensitivity reactions.

TECK: Thymus-expressed chemokine.

Tetramer: Biotinylated MHC class I, or class II, molecule complexed to peptide and bound to labeled avidin. Used to determine the presence of peptide-specific T cells.

TGF: Transforming growth factor.

TH: T helper cells ($CD4^+$ T cell; see also Helper cells).

Thy: A cell surface antigen of mouse T cells; there are several allelic variants of this marker.

TNF: Tumor necrosis factor.

Tolerance: A state of specific immunological unresponsiveness.

Transformation (blastic): Morphological changes in a lymphocyte associated with the onset of cell division.

Transplantation antigens: See MHC.

Wiskott-Aldrich syndrome: A sex-linked, inheritable, recessive, combined immune deficiency in which IgM antibody production and cellular immune reactions are impaired.