Lymphology 10 (1975) 85-93

Immunological Identification of Human Lymphoid Cell Populations

Nelson F. Mendes

Departamento de Microbiologica, Imunologia e Parasitologia da Escola Paulista de Medicina e Departamento de Clinica Medica da Faculdade de Medicina da Universidade de Sao Paulo

Summary

Lymphocytes can be subdivided into two major groups according to origin and function (1, 2).

T lymphocytes are thymus dependent and are responsible for cell-mediated immunity while the B lymphocytes represent precursors of immunoglobulin-producing plasma cells and give rise to humoral immunity. Considerable knowledge concerning the biological properties of T and B lymphocytes and their mutual interaction has been obtained in the last few years. Recent data also demonstrate further heterogeneity within each of the two main lymphocyte populations.

Certain lymphoid cells are capable of mediating cytotoxicity against target cells coated with specific antibody. These cells are termed K cells, and although some evidence seems to suggest that they comprise a subpopulation of B lymphocytes, their nature and localization in lymphoid organ is not well known.

This article will attempt to cover the methodologies involved in assays of T, B, and K cells. The methods to be discussed are used to detect the presence of different lymphoid cell populations; the markers used are not necessarily related to cell function.

Rosette Formation with Sheep Erythrocytes (E)

Lay et al., in 1971, first described, rosette formation between human lymphocytes and E as a probable T-cell marker (3). Other reports on the same phenomenon at the same time have not postulated its possible relationship with T cells (4, 5). Definite proof that E represents a valid T-cell marker was also obtained in our laboratory by studying the tissue distribution of lymphoid cells with membrane receptors for E in cryostat sections (6). A massive adherence of E to the thymus and to thymus-dependent areas of lymph nodes and spleen could be demonstrated. Recently, these studies have been extended to other human lymphoid organs (7).

The identification of T cells by rosette formation with E has been widely used. A large number of publications are available which deal with technical parameters, biological significance, and clinical applications (survey: 8-10).

In our laboratory, the method has been applied in a variety of diseases (11-18).

The method of rosette formation that we are currently using is a modification of the one originally described by *Lay* et al. (3). A detailed description was given in recent papers (14, 19). Basically, the rosettes are obtained by mixing purified human lymphocytes with E and incubating the mixture at 4°C after favoring the contact by centrifugation. The cells are then resuspended gently with a Pasteur pipette and the rosettes counted in a hemocytometer. Only lymphocytes having 3 or more red cells around then are recorded as rosette-forming cells. In addition to sheep erythrocytes, erythrocytes other animal species such as pig (3, 5, 20), dog (20, 21), goat (21), horse, and burro (22) form rosettes with human lymphocytes. However, these rosettes are formed in smaller numbers than E rosettes and are less stable (20).

A very small number of E-rosetting cells carry surface markers usually found on B cells, e.g., immunoglobulin, Fc, or C3 receptors (19, 23, 24). Furthermore, cells stimulated by mitogens or allogeneic lymphocytes retain their capacity to bind E (25-28). Finally, it appears that rosette formation with E is an easy method for separating lymphocyte populations by gradient centrifugation (29).

* Supported by Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, Coordenadoria do Aperfeicoamento do Pessoal de Nivel Superior e Fundaçao de Amparo a Pesquisa do Estado de Sao Paulo.

Technical Aspects

The technical aspects of the interaction between human T lymphocytes and E have been studied in detail in our laboratory and at Duke University (29). The rosette phenomenon is extremely unstable and sensitive to physical changes. The number of rosettes in normal blood varies widely depending on the method used. Our present technique (19) usually gets figures over 60%. The main steps of the current method are as follows:

1. Lymphocyte separation on Ficoll-Hypaque gradient;
2. 0.5% E suspension in HBSS (100 μl);
3. 3 x 10^6 lymphocytes/mm^3 in HBSS (60μl);
4. AB serum absorbed with E (40 μl);
5. Mixture of 2, 3, 4 and 4 in 6 x 50 mm glass tubes;
6. 5 minutes incubation at 37°C;
7. Centrifugation (200 g, 5 min);
8. 1 hour incubation at 4°C;
9. Gentle resuspension with a Pasteur pipette after the addition of 1 drop of 0.33% methylene blue;
10. Reading in hemocytometer;
11. Results expressed in percentage and in absolute number/mm^3 (in reference to lymphocyte differential cell count).

In our experience, addition of serum to the medium has a stabilizing effect which increases the number of cells detected.

The temperature is a crucial factor since there is no rosette formation at 0°C or 37°C (29). Most authors employ a two-step incubation (37°C for 5 to 60 minutes and 4°C for at least 60 minutes). The temperature of the second step may be from 4°C to 25°C (29). At temperatures over 37°C, rosettes are not-formed since the receptors for E are released from the cell surface by heating (30, 31). Treatment of lymphocytes with certain enzymes such as neuraminidase or papain increase rosette formation (27, 32, 33) while other enzymes apparently destroy the receptor (trypsin, phospholipase A) (33, 34). A disadvantage of neuraminidase treatment is that some B cells may form rosettes with E by exposing "hidden" receptors. Rosette formation can also be abolished by treating E with trypsin or papain (35, 36). The binding of E to T cells can be strengthened by treating the red cells with neuraminidase (35-37) or by adding 2-amino-ethyisothiouronium (38).

The optimal pH for rosette formation is between 7 and 8 (34). Metabolic inhibitors, drugs increasing the levels of cyclic AMP and ADTA, cause a decrease in rosette formation (34, 39-42).

Biological Aspects

The receptor for E can be recovered in a soluble from the supernatant of heated (45°C) lymphocytes, normal human serum, and from preparations containing transfer factor and thymosin. This soluble receptor can restore rosette formation with E in previously heated lymphocytes. In addition it als binds to E; the complex formed (ER) is able to form rosettes with lymphocytes deprived of their receptors by previous heating (30, 31).

Incipient studies on the partial purification and characterization of the receptor have been made, but its chemical nature is not known (43-46). The structures on the red blood cell membrane which bind to the lymphocyte are likewise not characterized.

Rosette formation can be inhibited by treating the lymphocytes with heterologous antilymphocyte sera (47). Experiments from our laboratory have shown that rabbit antihuman brain serum, in spite of its lymphocytotoxic activity, does not inhibit rosette formation (48).

We have demonstrated the presence of antibodies cytotoxic for T cells which are capable of inhibiting rosette formation in the following types of antisera (48):

1. Rabbit antihuman thymus;
2. Sheep anti-T-cell E rosettes purified in a Ficoll-Hypaque gradient;
3. Sheep anti-T-cell receptor for E.

This antiserum, specific for the T-cell receptor, could be obtained by immunizing sheep with E sensitized with the supernatant from heated (45°C) human thymus cell suspension. The soluble receptors (R) for E present in this preparation can be specifically adsorbed onto the red blood cells; the ER complexes formed

are washed and used to immunize sheep. Since the red blood cells are autologous, the antibodies formed will react only with the receptors. The above antisera, with antireceptor activity are cytotoxic for T cells, inhibit rosette formation, are able to aglutinate ER complexes, and can also be used to identify T cells by immunofluorescence. The antireceptor antibody also reacts in vitro with the receptor in a soluble form since normal human serum dialysate, the supernatant from heated lymphocytes, and preparations containing transfer factor neutralize the antibody activity against the receptor as evaluated by cytotoxicity, inhibition of rosette formation, and ER aglutination (48).

Studies recently carried out in our laboratory have shown a biological activity possessed by this receptor. Preparations containing this receptor in a soluble form such as supernatant from heated lymphocytes, normal human sera dialysate, and transfer factor have a potent chemotactic activity for granulocytes (49). This chemotactic activity is lost by absorption of these preparations with E. The chemotactic activity of preparations containing transfer factor was already known (50) and, therefore, probably is associated with the presence of the soluble receptor for E. The chemotactic activity of the soluble receptor for E is not removed by absorption with rabbit erythrocytes or with trypsinized sheep erythrocytes since the receptors do not bind to these cells. Other chemotactic preparations such as endotoxin-activated human serum which contain C3a or C5a do not loose their activity by absorption with E (49).

Anti-T-Cell Antibodies

Antisera specifically reactive with human T cells can be obtained by immunization of several animal species with thymus cells, peripheral-blood T cells, T leukemia cells, T-cell lines, and brain (51-55). In addition, as mentioned above, anti-T-cell antibodies directed against the receptor for E can be obtained by immunizing sheep with E-soluble receptor for E (ER) complexes (48). Heterologous antisera with anti-T-cell activity usually include antibodies which are species specific and lymphocyte specific. Absorption with insolubilized human immunoglobulin, erythrocytes, liver, and kidney can be used to remove species-specific antibodies. The antibodies can be rendered specific for T cells by further absorption with purified B cells.

A specific anti-T-cell sera should react with the majority of thymus cells and give a "plateau" detecting 70% to 80% of peripheral blood lymphocytes, which are the E-rosette positive cells.

The tests commonly used to identify T cells using specific antibodies have been cytotoxicity and immunofluorescence, both direct and indirect.

Measles Virus Receptors

Recently, *Valdimarsson* et al. (56) showed that human T cells have membrane receptors for measles virus. T cells can be enumerated by this technique by rosette formation with cells infected with measles virus. The selectivity of measles virus for T cells corresponds with the capacity of this virus to impair T-cell-mediated immune responses (57).

Helix Pomatia A Hemagglutinin Receptors

Neuraminidase-treated human T lymphocytes have receptors for the A hemagglutinin of the snail *Helix pomatia* (HP) (58). The reaction of HP with T lymphocytes can be visualized with fluorescein-labeled HP (58) or, quantitatively, by determining the binding of ^{125}I-HP to the cells (59).

Identification of B Lymphocytes

The major procedures currently in use to identify human B cells are immunoglobulin staining by fluorescence, determination of complement receptors, and determination of Fc receptors. Other methods include the detection of Epstein-Barr virus receptors, rosette formation with mouse erythrocytes, and anti-B-cell antibodies.

Surface Immunoglobulin

The presence of intrinsic surface membrane immunoglobulin on the B cell is most common-

ly demonstrated by direct immunofluorescence. However, false positive surface staining may occur due to the binding of immune complexes or immunoglobulin aggregates to Fc receptors and due to the reaction of auto-anti-lymphocyte antibodies. These antibodies can be found in a variety of diseases including systemic lupus erythematosus, rheumatoid arthritis, certain malignancies and certain infectious diseases. Usually the activity of these antibodies increase with cold, and therefore, they can be eluted at 37°C for 45 to 60 minutes. These antibodies are primarily of the IgM class and, to a lesser extent, of the IgG class.

In order to diminish these sources or error, the detection of immunoglobulin associated with the cell membrane shoud be performed by using F (ab') 2 fragments of the fluorescent antihuman immunoglobulin, immune complexes formed by the reaction with minute amounts of serum immunoglobulins will not bind to Fc receptors. The most convenient methods for preparations of reagents, cell separation, and staining for the detection of immunoglobulin-bearing cells have been described in detail recently by *Winchester* and *Fu* (62). The values for IgG-bearing and IgA-bearing cells vary from 0% to 1%; those bearing IgD and/or IgM range from 3% to 18% approximately (62).

Complement Receptors

Human complement receptor lymphocytes (CRL) have receptors for C3b (immune adherence receptor) and/or for C3d. Most CRL have both types of receptors. These two types of receptors cap independently and interact with different regions of C4 or C3 molecules (63, 64). The C3b receptor is also present in human erythrocytes, granulocytes, and monocytes; it reacts with C4b or the C3c region of C3b. The C3b receptor can also be detected in monocytes and eosinophils (65, 66).

An average of 12% CRL (range 6% to 18%) in normal peripheral blood lymphocytes has been usually reported using EAC14, EACl-3b or EACl-3d (63, 67, 68).

Several methods have been reported for detecting CRL. One of the most common utilizes erythrocytes (E) sensitized with antibody (A) and complement (yields EAC which forms rosettes with CRL). Sheep E should not be used to prepared EAC since they can form rosettes with human T cells directly due to the receptor for E (29). Red blood cells from any animal species unable to interact with human T cells can be used to prepare the EAC reagent.

In our experience as well as in other laboratories, human erythrocytes (HE) sensitized with IgM rabbit antibody (A) and complement (C) have been used successfuly as an indicator for CRL (29, 36). The immune adherence receptors present in HE do not interfere with the detection of CRL.

In 1974 we introduced a new method for detecting CRL, which uses zymosan-C3 complexes (ZC). Zymosan activates the complement system by the alternate pathway so that there is no need of the antibody. Another advantage is that any source of complement can be used since the zymosan particles are not lysis-passive (19, 69). Using a mixture of ZC and E, B, and T, human cells can be detected simultaneously in the same suspension. The ZC method is the quickest and most practical way of detecting CRL. This method has been recently confirmed by another laboratory (70).

The main steps of the HEAC and ZC methods are as follows:

1. Lymphocyte separation on Ficoll-Hypaque gradient;
2. 0.5% HEAC or ZC (10^8 ZC particles/ml) suspension in HBSS (100 μl)
3. 2 x 10^6 lymphocytes/mm^3 in HBSS (100 μl);
4. Mixture of 2 and 3 in 6 x 50 mm glass tube;
5. Centrifugation (200 g, 5 min);
6. Gentle resupension with a Pasteur pipette after the addition of 1 drop of 0.33% methylene blue;
7. Reading in hemocytometer;
8. Results expressed in percentage and in absolute number/mm^3 (in relation to lymphocyte differential cell count).

CRL have also been detected with soluble radiolabeled immune complexes (71) and

soluble complement components labeled by fluorescence (63, 72) or radioactivity (72). These methods have a high specificity and can be used for double-label studies.

The first important indication that human CRL are B cells was demonstrated in our laboratory. Here it was shown that the population of E rosette-forming cells and CRL do not overlap and that the distribution of CRL corresponds to thymus-independent areas of lymphoid tissues (6).

It has recently been demonstrated that when F(ab')2 fragment of anti-immunoglobulin are used, only 50% to 70% of CRL are double-labeled. It was also shown that most of the CRL lacking surface immunoglobulin contained Fc receptors. In addition, 15% to 30% of CRL lack both immunoglobulin and Fc receptors (73). Therefore, it still must be established whether or not all human CRL with Fc receptors might be K cells, responsible for antibody-dependent cytotoxicity or the "third population" described by *Winchester* et al. (60).

Fc Receptors

Early studies suggested a correspondence between B cells and the presence of Fc receptors (74). More recently, however, these receptors have also been shown in other lymphocyte subpopulations (75).

The detection of cells bearing Fc receptors can be achieved by rosette formation with erythrocytes (E) sensitized by IgG antibodies (A) and by the binding of fluorescein or ^{125}I-labeled, heat-aggregated or antigen-complexed IgG (74-77). EA rosette-forming cells were reported to be mostly monocytes and B lymphocytes with surface membrane immunoglobulin or to belong to a third lymphocyte population (K cells) which lacked other membrane markers and possessed "high affinity" Fc receptors (78).

Human Fc receptor-bearing cells can be detected using human-indicator erythrocytes (OR_1R_2 cells) sensitized with anti-Rh (anti-CD) isoantibodies of the Ripley (Ri) type (79). Another current EA technique uses chicken erythrocytes coated with rabbit Ig 7S antibodies (78).

Epstein-Barr Virus Receptors

Epstein-Barr virus (EBV) appears to bind to B cells with the membrane phenotype SmIg+, C3+ (80, 81). Recently, it has been shown that the EBV binding sites and C3 receptors are probably part of the same molecular structure on B cells (82). The assays currently used for virus receptors have been recently reviewed (83).

The observation of EBV receptors are relevant to understand the cellular events involved in infectious mononucleosis and other lymphoproliferative diseases. Its usefulness as a marker of B cells in normal blood, however, is limited

Mouse Erythrocyte Receptors

A small proportion (5% to 11%) of normal human peripheral blood lymphocytes and a large proportion of lymphocytes from patients with chronic lymphocytic leukemia form rosettes with mouse erythrocytes (84). Recent evidence has shown that these lymphocytes probably represent a subpopulation of B cells (85).

Anti-B-Cell Antibodies

Heterologous antisera reacting against B cells can be obtained by immunization with peripheral-blood B lymphocytes, B chronic lymphocytic leukemia cells, and B lymphoid cells lines. These antisera can be rendered B cell specific by absorption with human erythrocytes, liver, kidney T cells, and insolubilized serum immunoglobulins (86).

Recently, the term HL-B has been applied to a group of alloantigens recognized by pregnancy sera and selectively expressed on B lymphocytes (87, 88). These antigens appear to be closely related to the Ia system of the mouse. Many pregnancy sery contain both HL-A and HL-B antibodies. The former can be removed by absorption with T cells or platelets. HL-B antibodies can be detected either by inhibition of mixed lymphocyte reactions, cytotoxicity of enriched B cell suspension, or indirect immunofluorescence.

Identification of B and T Lymphocytes in Tissue Sections

B and T cells can be identified in tissue sections by the adherence of HEAC or ZC and E (6, 7, 12, 19).

Our current technique uses 6 μm thick cryostat sections which are dried at room temperature for 10 minutes and then covered with one drop of either E or HEAC 0.5% or ZC (10^8/ml) suspension in HBSS. The sections are incubated at 37°C for 15 minutes and then for 1 hour at 4°C in a moist chamber. The excess indicator is removed by placing the sections over slaine (0.15 M sodium chloride) at 4°C for 20 minutes, thereby allowing the nonadherent erythrocytes or ZC to settle by gravity. The slides are then dried, fixed with absolute methanol, and stained with hematoxylin-eosin. The method can be used to study the distribution of T and B cells in lymphoid organs and in inflamatory infiltrates.

Identification of K Cells

Cells with a lymphocytic appearance and able to mediate antibody-dependent cytotoxicity of target cells are called K cells. These cells have receptors with strong affinity for Fc of IgG; the interaction between target cell-bound IgG and the Fc receptors may trigger the cytotoxic effect (89).

It seems that the majority of the peripheral T lymphocytes do not desplay K cell activity (90).

Usually a K cell assay system consists of purified lymphocytes, ^{51}Cr-labeled chicken erythrocytes serving as target cells, and hyperimmune rabbit anti-chicken erythrocytes antibodies (IgG) as the inducing agent (89).

B cells with high concentrations of SIg+ which have Fc receptors with low affinity are inactive in the K-cell assay. Some of the Fc+ cells seem to have E receptors and are probably also active in the K-cell assay (89).

References

1 *Cooper, M.D., D.Y. Perey, R.D.A. Peterson, A.E. Gabrielsen, R.A. Good:* The two-component concept of the lymphoid system. In: Immunologic Deficiency Diseases in Man. Birth Defects Original Article Series, vol. 4, ed. Good, R.A. Bergsma, D. p. 7. National Foundation Press, New York 1968

2 *Greaves, M.F., J.J.T. Owen, M.C. Raff:* T and B lymphocytes. Origins, properties and role in immune response. American Elsvier, New York 1973

3 *Lay, W.H., N.F. Mendes, C. Bianco, V. Nussenzwig:* Binding of sheep red blood cells to a large population of human lymphocytes. Nature 230 (1971) 531-532

4 *Coombs, R.R.A., B.W. Gurner, A.B. Wilson, G. Holm, B. Lindgren:* Rosette formation between human lymphocytes and sheep red cells not involving immunoglobulin receptors. Int. Arch. Allergy 39 (1970) 658-663

5 *Brain, P., J. Gordon, W.A. Willets:* Rosette formation by peripheral lymphocytes. Clin. exp. Immunol. 6 (1970) 681-688

6 *Silveira, N.P.A., N.F. Mendes, M.E.A. Tolnai:* Tissue localization of two populations of human lymphocytes distinguished by membrane receptors. J. Immunol. 108 (1972) 1456-1460

7 *Mello, J.F., S. Levy, C.A.R. Freire, N.F. Mendes:* Localization of T and B lymphocytes in human adenoid, tonsil, appendix and Peyer's patches. Allergol. Immunopathol. (in press)

8 *Bach, J.F.:* Evaluation of T-cells and thymic serum factors in man using the rosette technique. Transplant. Rev. 16 (1973) 196-217

9 *Jondal, M.:* SRBC rosette formation as a human T lymphocyte marker. Scand. J. Immunol. 5, Suppl. 5 (1976) 69-76

10 *Scheinberg, M.A., N.F. Mendes, S. Kopersztych, E.S. Cathcart:* Clinical applications of T, B and K cell determinations in rheumatic diseases. A review. Seminars in Arthritis and Rheumatism 6 (1976) 1-18

11 *Mendes, N.F., C.C. Musatti, M.E.A. Tolnai:* Lymphocyte membrane markers in cells from patients with leukemia and lymphoma. Int. Arch. Allergy 46 (1974) 695-706

12 *Mendes, N.F., S. Kopersztych, N.G.S. Mota:* T and B lymphocytes in patients with lepromatous leprosy. Clin. exp. Immunol. 16 (1974) 23-30

13 *Galvao, M.M., E. Sabbaga, Z.F. Peixinho, C.C. Musatti, N.F. Mendes:* Immunossupressive effect of intra-lymphatic irradiation. Allergol. Immunopathol. 3 (1975) 299-308

14 *Mendes, N.F.:* Lymphocytes and lymph nodes in patients with paracoccidioidomycosis. In: Mycoses. Proceedings of the Third International Conference on the Mycosis. Pan American Health Organization. Scientific publication No. 304 (1976) 30-35

15 *Kopersztych, S., M.T. Rezkallah, S.S. Miki, C,K. Naspitz, N.F. Mendes:* Cell-mediated immunity in patients with carcinoma. Correlation between clinical stage and immunocompetence. Cancer 38 (1976) 1149-1154

16 *Maciel, R.M.B., S.S. Miki, W. Nicolau, N.F. Mendes:*

Peripheral blood T and B lymphocytes *in vitro* stimulation with phytohemagglutinin and sensitization with 2,4 dinitrochlorbenzene in Graves' diseases. J. Clin. Endocrinol. Metab. 42 (1976) 583-587

17 *Musatti, C.C., M.T. Rezkallah, E. Mendes, N.F. Mendes: In vivo* and *in vitro* evaluation of cell-mediated immunity in patients with paracoccidioidomycosis. Cell. Immunol. 24 (1976) 365-378

18 *Montufar, O.M.B., C.C. Musatti, E. Mendes, N.F. Mendes:* Cellular immunity in chronic Chagas' disease. J. Clin. Microbiol. (in press)

19 *Mendes, N.F., S.S. Miki, Z.F. Peixinho:* Combined detection of human T and B lymphocytes by rosette formation with sheep erythrocytes and zymosan-C3 complexes. J. Immunol. 113 (1974) 531-536

20 *Bach, J.F., J. Dormont:* Further developments of the rosette inhibition test for the testing of anti human lymphocyte serum. Transplantation 11 (1971) 96-100

21 *Ross, G.D., M.J. Polley, H.M. Grey:* Evidence for two distinct complement receptors on the surface of human lymphocytes. Fed. Proc. 32 (1973) 992

22 *Whittingham, S., I.R. Mackay:* Rosette formation by human thymocytes. Cell. Immunol. 6 (1973) 362-367

23 *Dickler, H.B., N.F. Adkinson, W.D. Terry:* Evidence for individual human peripheral blood lymphocytes bearing both B and T cell markers. Nature (Lond) 247 (1974) 213-215

24 *Chiao, J.W., V.S. Pantic, R.A. Good:* Human lymphocytes bearing both receptors for complement components and SRBC. Clin. Immunol. Immunopathol. 4 (1975) 545-556

25 *Mendes, N.F., M.J. Zenha, C.C. Musatti, C.K. Naspitz:* Lymphocyte membrane receptors in cultures stimulated by mitogens. Cell. Immunol. 12 (1974) 331-337

26 *Greaves, M.F., G. Janossy, M. Doenhoff:* Selective triggering of human T and B lymphocytes *in vitro* by polyclonal mitogens. J. exp. Med. 140 (1974) 1-18

27 *Jondal, M.:* Surface markers on human B and T lymphocytes. IV. Distribution of surface markers on resting and blast-transformed lymphocytes. Scand. J. Immunol. 3 (1974) 749-755

28 *Kreeftenberg, J.G., M.F. Leerling, H.G. Loggen:* B and T-cell markers on human lymphoblasts after stimulation with mitogen or antigens. Clin. exp. Immunol. 22 (1975) 121-125

29 *Mendes, N.F., M.E.A. Tolnai, N.P.A. Silveira, R.S. Metzgar, R. Gilbertsen:* Technical aspects of rosette tests used to detect human complement receptor (B) and sheep erythrocyte binding (T) lymphocytes. J. Immunol. 111 (1973) 860-867

30 *Mendes, N.F.:* T-cell markers. In Brent, L. & Holborow, J. (eds.). Progress in Immunology II vol. 3, p. 299. North-Holland American Elsevier, 1974

31 *Mendes, N.F., P.J. Saraiva, O.B.O. Santos:* Restorative effect of normal human serum transfer factor and thymosin on the ability of heated lymphocytes to form rosettes with sheep erythrocytes, Cell. Immunol. 17 (1974) 560-566

32 *Bentwich, Z., S.D. Douglas, E. Skutelsky, H. Kunkel:* Sheep red blood cells binding to human lymphocytes treated with neuraminidase; enhancement of T cell binding and identification of a subpopulation of B cells. J. exp. Med. 137 (1973) 1532-1537

33 *Chapel, H.M.:* The effects of papain, trypsin and phospholipase A on rosette formation. Transplantation 15 (1973) 320-325

34 *Jondal, M., G. Holm, H. Wigzell:* Surface markers on human B ant T lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. J. exp. Med. 136 (1972) 207-215

35 *Weimer, M.S., C. Bianco, V. Nussenzweig:* Enhanced binding of neuraminidase-treated sheep erythrocytes to human T lymphocytes. Blood 42 (1973) 939-946

36 *Gilbertsen, R.B. R.S. Metzgar:* Human T and B lymphocyte rosette tests. Effect of enzymatic modification of sheep erythrocytes (E) and the specificity of neuraminidase treated. E. Cell. Immunol. 24 (1976) 97-108

37 *Galili, U., M. Schlesinger:* The formation of stable E rosettes after neuraminidase treatment of either human peripheral blood lymphocytes or of sheep red blood cells. J. Immunol. 112 (1974) 1628-1634

38 *Kaplan, J., R. Masriangelo, W.D. Peterson:* Childhood lymphoblastic lymphoma, a cancer of thymus derived lymphocytes. Cancer Res. 34 (1974) 521-525

39 *Froland, S.S.:* Binding of sheep erythrocytes to human lymphocytes. A probable marker of T lymphocytes. Scand. J. Immunol. 1 (1972) 269-280

40 *Chisari, F.V., T.S. Edgington:* Human T lymphocyte E rosette function. I. A process modulated by intracellular cyclic AMP. J. exp. Med. 140 (1974) 1122-1126

41 *Lohrmann, H.P., L. Novikovs:* Rosette formation between human T lymphocytes and unsensitized rhesus monkey erythrocytes. Clin. Immunol. Immunopathol. 3 (1974) 99-111

42 *Grieco, M.H., I. Siegel, Z. Goel:* Modulation of human T lymphocyte rosette formation by autonomic agonists and cyclic nucleotides. J. Allergy Clin. Immunol. 58 (1976) 149-159

43 *Owen, L.O., M.W. Fanger:* Studies on the human T lymphocyte population. I. The development and characterization of a specific anti human T-cell antibody. J. Immunol. 113 (1974) 1128-1137

44 *Owen, L.O., M.W. Fanger:* Studies on the human T lymphocytes. II. The use of a specific T-cell antibody in the partial isolation and characterization of the human receptor for sheep red blood cells. J. Immunol. 113 (1974) 1138-1144

45 *Owen, F.L., M.W. Fanger:* Studies on the human T lymphocytes population. III. Synthesis and release of the lymphocytes receptor for sheep red blood cells by stimulated human T lymphoblasts. J. Immunol. 115 (1975) 765-770
46 *Pyke, K.W., G.A. Rawlings, E.W. Gelfand:* Isolation and characterization of the sheep erythrocyte receptor in man. J. Immunol. 115 (1975) 211-215
47 *Wortis, H.H., A.G. Cooper, M.C. Brown:* Inhibition of human lymphocyte resetting by anti-T sera. Nature New Biol. 243 (1973) 109-110
48 *Mendes, N.F., H.H.A. Cintra:* Manuscript in preparation
49 *Mendes, N.F., E. Mendes:* Transfer factor. Biological properties and therapeutic uses. In: Proceedings of the IX International Congress of Allergology, Excerpta Medica. (in press)
50 *Gallin, J.I., C.H. Kirkpatrick:* Chemotactic activity in dialyzable transfer factor. Proc. Nat. Acad. Sci. USA 71 (1974) 498-502
51 *Yata, J., I. Tsukimoto, T. Tachibana:* Human lymphocyte subpopulations. Human thymus-lymphoid tissue (HTL) antigen-positive lymphocytes forming rosettes with sheep erythrocytes and HTL antigen-negative lymphocytes interacting with antigen-antibody-complement complexes. Clin. exp. Immunol. 14 (1973) 309-317
52 *Ablin, R.J., A.J. Morris:* Thymus-specific antigens on human thymocytes and on thymic-derived lymphocytes. Transplantation 15 (1973) 415-417
53 *Takada, A., Y. Takada, U. Ito, J. Minowada:* Shared antigenic determinants between human brain and human T-cell line. Clin. exp. Immunol. 18 (1974) 491-498
54 *Brouet, J.C., H. Toben:* Characterization of a subpopulation of human T lymphocytes reactive with a heteroantiserum to human brain. J. Immunol. 116 (1976) 1041-1044
55 *Small, P., N. Kashiwagi, P.F. Kohler:* Studies of the sheep red blood cell receptor using anti-lymphocyte antibodies. Cell. Immunol. 25 (1976) 302-308
56 *Valdimarsson, H., G. Agnarsdottir, P.J. Lachmann:* Measles virus receptor on human T lymphocytes. Nature 255 (1975) 554-556
57 *Notkins, A.L., S.E. Mergenhagen, R.J. Howard:* Effect of virus infections on the function of the immune system. Ann. Rev. Micr. 24 (1970) 525-538
58 *Hammarström, S., P. Hellström, P. Perlmann, M.L. Dillner:* A new surface marker on T lymphocytes of human peripheral blood. J. exp. Med. 138 (1973) 1270-1275
59 *Dillner, M.L., S. Hammarström, P. Parlmann:* The lack of mitogenic response of neuraminidase treated and untreated human blood lymphocytes to divalent, hexavalent or insoluble Helix pomatia A hemagglutinin. Exp. Cell. Res. 96 (1975) 374-382
60 *Winchester, R.J., S.M. Fu, T. Hoffman, H.G. Kunkel:* IgG on lymphocyte surfaces; technical problems and the significance of a third cell population. J. Immunol. 114 (1975) 1210-1212
61 *Winchester, R. Jr., J.B. Winfield, F. Siegal, P. Wernet, Z. Bentwich, H.G. Kunkel:* Analyses of lymphocytes from patients with rheumatoid arthritis and systemic lupus erythematosus. J. Clin. Invest. 54 (1974) 1082-1092
62 *Winchester, R.Jr., S.M. Fu:* Lymphocyte surface membrane Immunoglobulin. Scand. J. Immunol. 5, Suppl. 5 (1976) 77-82
63 *Ross, G.D., M.J. Polley:* Specificity of human lymphocyte complement receptors. J. exp. Med. 141 (1975) 1163-1180
64 *Ross, G.D., M.J. Polley, E.M. Rabellino, H.M. Grey:* Two different complement receptors on human lymphocytes. One specific for C3b and one specific for C3b inactivator-cleaved C4b. J. exp. Med. 138 (1973) 798-811
65 *Reynolds, H.Y., J.P. Atkinson, H.H. Newball, M.M. Frank:* Receptors for immunoglobulin and complement on human alveolar macrophages. J. Immunol. 144 (1975) 1813-1819
66 *Grupta, S., G.D. Ross, R.A. Good, F.P. Siegal:* Surface characteristics of human eosinophils. J. Allergy Clin. Immunol. 57 (1976) 189-266
67 *Bokisch, V.A., A.T. Sobel:* Receptor for the fourth component of complement on human B lymphocytes and cultured human lymphoblastoid cells. J. exp. Med. 140 (1974) 1336-1347
68 *Pincus, S., C. Bianco, V. Nussenzweig:* Increased proportion of complement-receptor lymphocytes in the blood of patients with chronic lymphocytic leukemia. Blood 40 (1972) 303-310
69 *Kajdacsy-Balla, A., N.F. Mendes:* Rosette formation between human B lymphocytes and zymosan-C3 complexes using different complement sources. J. Immunol. Methods 9 (1976) 205-209
70 *Huber, Ch., H. Wigzell:* A simple rosette assay for demonstration of complement receptor sites using complement-coated zymosan beads. Eur. J. Immunol. 5 (1975) 432-435
71 *Miller, G.V., P.H. Saluk, V. Nussenzweig:* Complement dependent release of immune complexes from the lymphocyte membrane. J. exp. Med. 138 (1973) 495-507
72 *Teophilopoulos, A.N., V.A. Bokish, F.J. Dixon:* Receptor for soluble C3 and C3b on human lymphoblastoid (Raji) cells. Properties and biological significance. J. exp. Med. 139 (1974) 696-711
73 *Ross, D.G., M.J. Polley:* Assay for the two different types of lymphocyte complement receptors. Scand. J. Immunol. 5, Suppl. 5 (1976) 99-111
74 *Basten, A., J.F.A.P. Miller, J. Sprent, J. Pye:* A receptor for antibody on B lymphocytes. I. Method of detection and functional significance. J. exp. Med. 135 (1972) 610-626
75 *Grey, H.M., C.L. Anderson, C.H. Heusser. J.T. Kurnick:* Fc receptors on lymphocytes other than B cells in mouse and man. In: Membrane

receptors of lymphocytes pp 185-192. Eds. M. Seligman, J.L. Preud'homme and F.M. Kourilsky. North-Holland/American Elsevier, Amsterdam 1975

76 *Dickler, H.G., H.G. Kunkel:* Interaction of aggregated γ-globulin with B lymphocytes. J. exp. Med. 136 (1972) 191-196

77 *Fröland, S.S., J.B. Natvig:* Identification of three human lymphocyte populations by surface markers. Transplant. Rev. 16 (1973) 114-162

78 *Revillard, J.P., C. Samarut, G. Cordier, J. Brochier:* Characterization of human lymphocytes bearing Fc receptors with special reference to cytotoxic (K) cells. In: Membrane receptors of lymphocytes pp. 171-184. Eds. M. Seligman, J.L. Preud'homme and F.M. Kourilsky. North Holland/American Elsevier, Amsterdam 1975

79 *Natvig, J.B., S.S. Fröland:* Detection of a third lymphocyte-like cell type by rosette formation with erythrocytes sensitized by various anti-Rh antibodies. Scand. J. Immunol. 5, Suppl. 5 (1976) 83-89

80 *Greaves, M.F., G. Brown, A.B. Rickinson:* Barr virus binding sites on lymphocyte sub-populations and the origin of lymphoblasts in cultured lymphoid cell lines and in the blood of patients with infectious mononucleosis. Clin. Immunol. Immunopathol. 3 (1975) 514-524

81 *Jondal, M., G. Klein:* Surface markers on human B and T lymphocytes. II. Presence of Epstein-Barr virus receptors on B lymphocytes. J. exp. Med. 138 (1973) 1365-1378

82 *Jondal, M., G. Klein, M.B.A. Oldstone, V. Bokish, E. Yefenol:* Surface markers on human B and T lymphocytes. VIII. Association between complement and Epstein-Barr virus receptor on human lymphoid cells. Scand. J. Immunol. 5 (1976) 401-410

83 *Greaves, M.F.:* Virus receptor on lymphocytes. Scand. J. Immunol. 5, Suppl. 5 (1976) 113-123

84 *Stathopoulos, G., E.V. Elliot:* Formation of mouse or sheep red blood cells rosettes by lymphocytes from normal and leukaemic individuals. Lancet I (1974) 600-601

85 *Forbes, I.J., P.D. Zalewski:* A subpopulation of human B lymphocytes that rosette with mouse erythrocytes. Clin. exp. Immunol. 26 (1976) 99-107

86 *Aïuti et al.:* Identification, enumeration and isolation of B and T lymphocytes from human peripheral blood. International Union of Immunological Societies (IUIS). Report-July 1974. Clin. Immunol. Immunopathol. 3 (1975) 584-597

87 *Winchester, R.J., S.M. Fu, P. Wernet, H.G. Kunkel: B. Dupont, C. Jersild:* Recognition by pregnancy serums of non-HL-A alloantigens selectively expressed on B lymphocytes. J. exp. Med. 141 (1975) 924-929

88 *Mann, D.L., L. Abelson, S. Harris, D.B. Amos:* Detection of antigens specific for B lymphoid cultured cell lines with human alloantisera. J. exp. Med. 142 (1975) 84-89

89 *Perlmann, H., P. Pearlmann, G.R. Pape, G. Hallden:* Purification, fractionation and assay of antibody-dependent lymphocytic-effector cells (K cells) in human blood. Scand. J. Immunol. 5, Suppl. 5 (1976) 57-68

90 *Perlmann, P., H. Perlmann, H. Wigzell:* Lymphocyte mediated cytotoxicity *in vitro*. Induction and inhibition by humoral antibody and nature of effector cells. Transplant. Rev. 13 (1972) 91-114

F. Nelson, M.D. Mendes, Escola Paulista de Medicina, Dept. de Mierobiologie, Immunologia e Parasitologia, Caixa Postal 7144, Sao Paulo/Brasil