THE UNIVERSITY OF MANITOBA

SURFACE CHARGE PROPERTIES OF IMMUNOCYTES

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF IMMUNOLOGY

WINNIPEG, MANITOBA

SPRING, 1979

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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ACKNOWLEDGEMENT

The author wishes to express his sincere thanks and appreciation to Prof. A. H. Sehon for his introduction to the exciting field of immunology, and for his guidance and helpful suggestions during the course of this research and in the preparation of the thesis.

Particular thanks are due to Drs. I. Berczi, A. Froese, S. Fujimoto and P. Tsui for their helpful suggestions during this research, and to Drs. B. G. Carter, E. Sabbadini and F. C. Stevens for their helpful comments on this thesis.

The technical assistance of Mr. H. Sanderson for the preparation of some of the illustrations is appreciated.

The author is indebted to his wife, Linda, and daughter, Samantha, for their encouragement, patience and understanding.

This study was completed and the results presented to Drs. A. Froese, I. Berczi and B.G. Carter in 1974. However, for reasons beyond his control, the author could not complete the thesis manuscript before 1978 which is presented here in its final revised form.

This study was supported by a grant from the Medical Research Council of Canada to Prof. Sehon.

ABSTRACT

The aim of this investigation was to explore the possibility of identifying and isolating the subpopulation of immunocompetent cells of strain A mice, capable of binding to the hapten DNP, in terms of their unique cell surface charge properties. The animals were immunized with $(DNP)_{35}$-BSA and the electrophoretic mobilities (EPM) of their immunocytes, expressed in units of micron/sec/volt/cm (referred to hereafter as units) were measured in a specially designed cell electrophoresis chamber.

The EPM of lymph node cells of control animals peaked bimodally at the values of 0.8750 and 1.4000 units, while those of immunized animals peaked at the values of 0.8176 and 1.3417 units. Upon incubation with various samples of the synthetic mono α-DNP-poly-L-lysine (αDNP-PLL) of differing chain lengths, the cell population of the immunized animals originally possessing the low EPM value of 0.8176 unit exhibited a partially reduced electrophoretic mobility. A partially elevated electrophoretic mobility was observed in the low EPM cell population of the immunized animals when incubated with the ε-succinilated derivatives of various samples of the synthetic αDNP-PLL of differing chain lengths. Incubation of the lymphocytes of both control and immune animals with the poly-trimethyl ammonium derivatives of αDNP-PLL resulted in clumping of the cells, thus making it impossible to study the EPM of the cells interacting with this highly positively charged polymer.

To further characterize the low and high electrophoretic mobility cell populations, normal animals were treated with the immunosuppressive drug cyclophosphamide. The cell population with the low EPM cells was reduced in cell numbers, while the cell population with the high EPM cells remained relatively intact indicating that the low EPM cells were, in all probability, the more rapidly dividing B cells. On the other hand, treatment of cells of normal animals with AKR anti-C_3H Θ-antiserum or with neuraminidase resulted in the reverse effect, i.e. the cell population with the high EPM cells was substantially reduced; hence one may suggest that this population contained T cells.

Studies with DNP-Biogel affinity chromatography using the batch method for fractionation of hapten binding cells indicated that the method was not 'specific' enough due to the natural surface stickiness of the cells and the incapability of displacing all the antigen binding cells from the gel by hapten.

The practical use of cell electrophoresis as a tool for immunological investigations was further explored in studies of the Macrophage Electrophoretic Migration Inhibition Factor (MEMIF). Three specific antigenic systems were investigated, namely, the guinea pig MCD tumor system, the A/J mice 1509 tumor system and the synthetic DNP antigen system. In all cases, this factor(s) was released specifically by the

sensitized cells upon incubation with the immunizing antigens.

On the basis of all these data indicating that cells belonging to different classes of lymphocytes possess distinct charges, it is suggested that preparative cell electrophoresis may be developed into a powerful tool for the separation of immunocyte and,also possibly, for immunological diagnoses.

TABLE OF CONTENTS

SCOPE OF THE PRESENT INVESTIGATION 1

LITERATURE REVIEW 4

I. CELLS INVOLVED IN IMMUNE RESPONSES 4

(a) The Immunologically Competent Cells 4

(b) The Thymus and Central Lymphoid Tissue 6

(c) The Bone Marrow and Haemopoiesis 7

(d) The Macrophages 8

II. CELL COOPERATION IN THE IMMUNE RESPONSE 10

(a) Effects of the Carrier Portion of the Antigen Molecule on Immunogenicity (the Carrier Effect) 10

(b) Cell Cooperation in the Antibody Response .. 11

(c) Cell Cooperation in Cell Mediated Response . 12

(d) Antigenic Competition and Cell Cooperation . 12

(e) Immunological Memory and Cell Cooperation .. 12

III. THE MECHANISM OF CELL COOPERATION 14

(a) Concentration of Antigens 14

(b) Soluble Factor 14

(c) Accessory Cells 15

(d) Function of Immunocompetent Cells in genetic control of immune responsiveness 15

(e) Modulation of Immune Responses by Suppressor T Cells 16

IV. SURFACE CHARGE PROPERTIES OF IMMUNOCYTES 18

(a) Introduction 18

(b) Cell Surface Charge Properties and Immunocyte Subpopulations 18

(c) The Electrophoretic Differentiation of T and B Cells 19

(d) Differential Interaction of Anti-Lymphocyte Serum with Subpopulation of Immunocytes 21

(e) Free-flow Electrophoresis for the Elucidation of Immunological Events at the Cellular Level 21

(f) Macrophage Electrophoretic Mobility (MEM) Test for Lymphocyte Sensitization 23

(g) Studies related to Cell Electrophoresis ..

EXPERIMENTAL 26

INTRODUCTION 26

I. THE MICROELECTROPHORESIS CHAMBER 27

(a) Material 27

(b) Instrument 27

(c) Method 27

The Construction of the Micro-electrophoresis Chamber 27

The Construction of the Electrode Compartment 31

The Polymerization of the Acrylamide for Gel Formation 32

Circuit and Determination of Field Strength 32

The Determination of the Electrophoretic Mobility of Cells 34

Temperature 35

Setting up of the Instrument 36

Cleaning and Filling 36

Calibration of the Cell Micro-electrophoresis Unit 38

(d) Result and Conclusion 38

II. THE ELECTROPHORETIC MOBILITY PATTERNS OF CONTROL AND IMMUNIZED A/J MICE LYMPH NODE CELLS 44

(a) Material 44

(b) Method 44

The Preparation of DNP_{35}-BSA 44

Immunization Schedule 44

(c) Result and Conclusion 46

III. THE IDENTIFICATION OF THE HAPTEN SPECIFIC BINDING CELLS BY POLYIONIC CONJUGATES OF HAPTEN 52

(a) Material and Method 52

Synthesis of the Poly-L-Lysine Homo-Polymer 52

Synthesis of Univalent α DNP-ε-N-Carbobenzoxyl-Poly-L-Lysine (III) 52

Cleavage of Carbobenzoxyl Group 55

Succinic Acid Monomethyl Ester 55

Succinic Acid Chloride Monomethyl Ester 60

Synthesis of the Polysuccinilated Derivative of α DNP-PLL (III) 60

Synthesis of Poly-Trimethyl Ammonium Derivative of α DNP-PLL 61

Interaction of Immunocytes with DNP-Polymer Conjugates 61

(b) Result and Conclusion 62

The EPM of the Control A/J Lymph Node Cells treated with α DNP-$PLL_{15,000}$ Inhibition Test with α DNP-lysine HCl ... 62

The EPM of the Immunized A/J Lymph Node Cells treated with α DNP-$PLL_{15,000}$ Inhibition Test with α DNP-lysine HCl 62

The EPM of the Control A/J Lymph Node Cells treated with αDNP-$PLL_{150,000}$ Inhibition Test with αDNP-lysine HCl 65

The EPM of the Immunized Lymph Node Cells treated with αDNP-$PLL_{150,000}$ Inhibition Test with αDNP-lysine HCl 65

The EPM of the Control A/J Lymph Node Cells treated with the εsuccinilated derivative of αDNP-$PLL_{15,000}$ Inhibition Test with αDNP-lysine HCl 72

The EPM of the Immunized A/J Lymph Node Cells treated with the εsuccinilated derivative of αDNP-$PLL_{15,000}$ Inhibition Test with αDNP-lysine HCl (figure 13) 72

The EPM of the Control A/J Lymph Node Cells treated with the εsuccinilated derivative of αDNP-$PLL_{150,000}$ Inhibition Test with αDNP-lysine HCl 72

The EPM of the Immunized A/J Lymph Node Cells treated with εsuccinilated derivative of αDNP-$PLL_{150,000}$ Inhibition Test with αDNP-lysine HCl 77

The Interaction of the Control and Immunized A/J Lymph Node Cells with the Trimethyl Ammonium derivative of αDNP-PLL 77

IV. THE IDENTIFICATION OF THE HAPTEN BINDING CELL BY THE ADOPTED IMMUNOFLUORESCENCE TECHNIQUE 83

(a) Material and Method 83

The Preparation of α-N-DNP-lysine 83

The Synthesis of αDNP, ε-isothiocyanate fluorescein-lysine 83

The Modification of the Cell Electrophoresis Microscope 84

The Incubation of the Fluorescein hapten with the Cells 84

(b) Result and Conclusion 84

V. THE IDENTIFICATION OF THE HAPTEN BINDING CELL BY THE HAPTEN SPECIFIC BIOGEL AFFINITY COLUMN 88

(a) Material and Method 88

The Preparation of N-(2,4-dinitrophenyl)-6-Polyacrylamide-Hexamine 88

The Interaction of the DNP-Biogel with the Lymph Node Cells 89

The Dissociation of the Antigen Binding Cell with a Monovalent Hapten α DNP-lysine HCl 89

(b) Result and Conclusion 92

VI. THE IMMUNOLOGICAL SPECIFICITY OF THE HIGH ELECTRO-PHORETIC MOBILITY LYMPH NODE CELLS 93

(a) Material and Method 93

Preparation of Anti-θ Serum 93

(b) Result and Conclusion 93

VII. THE EFFECT OF NEURAMINIDASE ON THE CELL SURFACE CHARGE PROPERTIES 97

(a) Material and Method 97

The Treatment of Cells with Neuraminidase 97

(b) Result and Conclusion 97

VIII. THE EFFECT OF IMMUNOSUPPRESSIVE DRUG (CYCLO-PHOSPHAMIDE TO THE LYMPH NODE CELL DISTRIBUTION) 102

(a) Material and Method 102

The Preparation of the Animal 102

(b) Result and Conclusion 102

IX. MACROPHAGE ELECTROPHORETIC MIGRATION (MEM) TEST FOR LYMPHOCYTE SENSITIZATION 105

(a) Material and Method 105

The Treatment of Guinea Pig Peritneal Macrophages 105

The MEM Test 105

The <u>In-Vitro</u> System 106

The <u>In-Vivo</u> System 106

The Artificial System 109

(b) Result and Conclusion 111

GENERAL DISCUSSION 113

CONCLUSION 120

CLAIM OF ORIGINALITY 123

LIST OF ABBREVIATIONS...................................... 124

BIBLIOGRAPHY 125

LIST OF TABLES

Table I — Polyacrylamide Gel Composition 33

Table II — Electrophoretic Mobility of Human Red Cells at pH 7.4 and 25°C 41

Table III — The Macrophage Electrophoretic Migration (MEM) Test for Lymphocytes Sensitization (a) In Vitro System 107

Table IV — The Macrophage Electrophoretic Migration (MEM) Test for Lymphocytes Sensitization (b) In Vivo System 108

Table V — The Macrophage Electrophoretic Migration (MEM) Test for Lymphocytes Sensitization (c) Artificial System 110

LIST OF FIGURES

Figure 1 The microelectrophoresis chamber and its electrode compartments. 28

Figure 2 The electrophoretic móbility of human red blood cells versus the distance from the inner surface of the cell chamber in mm. The field strength was 80/35 volt/cm. The current was 5 milliamperes. The time of exposure was 10 seconds. 42

Figure 3 The EPM profile for lymph node cells of A/J mice immunized with FCA. The field strength was 80/35/v/cm and the current 5 milliamperes. 48

Figure 4 The EPM profile for lymph node cells of A/J mice immunized with 20 μgN αDNP$_{35}$-BSA in FCA. The field strength was 80/35/v/cm and the applied current 5 milliamperes. 50

Figure 5 Synthesis reagent. 53

Figure 6 Flow chart for synthesis of αDNP-PZLL. 56

Figure 7 Flow chart for synthesis of αDNP-PLL derivatives. 58

Figure 8 Histogram of percentage of control cell against EPM. Cells treated with αDNP-PLL$_{150,000}$. Inhibition test with αDNP-lysine HCl. Field strength 80/35 v/cm. Current applied 5 milliamperes. 63

Figure 9 Histogram of EPM versus percentage of immunized cells. Animal immunized with 20 μgN DNP$_{35}$-BSA at the foot pad and boosted after two weeks with the same dose subcutaneously. Cells have been incubated with the hapten αDNP-PLL$_{15,000}$. Inhibition test by αDNP-lysine HCl. 66

Figure 10 Histogram of EPM versus cell percentage. Cells are treated with αDNP-PLL$_{150,000}$. Inhibition test with αDNP-lysine HCl. Field strength 80/35 v/cm. Current applied 5 milliamperes......68

Figure 11 Histogram of EPM versus cell percentage (immunized). Cells are treated with αDNP-PLL$_{150,000}$. Inhibition test with αDNP-lysine HCl. Field strength 80/35 v/cm. Current applied is 5 milliamperes. 70

Figure 12 Histogram of the EPM versus cell percentage for the control animal lymph node cells. Cells are treated with ε succinilated derivatives of α DNP-PLL$_{15,000}$. Inhibition test by α DNP-lysine HCl. Field strength 80/35 v/cm. Current applied 5 milliamperes. 73

Figure 13 Histogram of the EPM of the immunized A/J lymph node cells treated with the ε succinilated derivative of α DNP-PLL$_{15,000}$. Inhibition test with α DNP-lysine HCl. Field strength 80/35 v/cm. Current applied 5 milliamperes. 75

Figure 14 Histogram of the control A/J lymph node cells. EPM versus percentage of cells. Cells treated with the ε succinilated derivative of α DNP-PLL$_{150,000}$. Inhibition test with α DNP-lysine HCl. Field strength 80/35 v/cm. Current applied 5 milliamperes. 78

Figure 15 The EPM of the immunized A/J lymph node cells treated with ε succinilated derivative of α DNP-PLL$_{150,000}$. Histogram EPM versus cell percentage. Field strength 80/35 v/cm. Current applied 5 milliamperes. 80

Figure 16 Synthesis of α DNP- ε -isothiocyanate fluorescein-L-lysine. 85

Figure 17 The EPM of cells versus percentage of cells histograms for the whole cell; fractionated and unfractionated cells with BioGel P6 >400 mesh. EPM taken in PBS, pH 7.2. Current applied 5 milliamperes. Field strength 80/35 v/cm. 90

Figure 18 The EPM of the A/J lymph node cells after treated with anti-θ serum (1 ml. of anti-θ serum, 10^6 lymph node cells and 2 ml. of fresh guinea pig complement) were incubated for 1 hour at room temperature. 94

Figure 19 The changes of the EPM of the control with neuraminidase. 98

Figure 20 The changes in EPM of the immune cell with neuraminidase. 100

Figure 21 The effect of the cyclophosphamide on the lymph node cell population *in vivo*. 103

LIST OF PLATES

Plate 1 Cell Microelectrophoresis Parts 30

Plate 2 The Microelectrophoresis Chamber 37

Plate 3 The tracts of Human Red Blood Cells covered under the influence of an electric field strength of 80/35 volt/cm. Scale, 1 Division = 10 microns.................... 40

SCOPE OF THE PRESENT INVESTIGATION

The immune response comprises all the phenomena that result from the interaction of cells of the immune system with antigen. As a consequence of such interactions, cells may become specialized to mediate cellular immune responses (such as delayed hypersensitivity, homograft rejection, or 'graft versus host' (GVH) reaction), or to synthesize and secrete one of several classes of immunoglobulins. Our current understanding of this complex interaction of cells, antibodies and other soluble mediators has been greatly aided by the use of animal models. With _in vitro_ culture techniques, information has been provided regarding the contribution of different lymphoid cell types to the immune responses.

Lymphocytes are the central cells for all immune responses and many attempts have, therefore, been made to isolate them. By the use of physical methods, many workers have attempted to separate populations of lymphoid cells into fractions displaying a limited degree of physical heterogeneity in size and charge, on the supposition that homogeneity in physical properties reflects singularity in biological function. For example, the widely used sedimentation velocity method leads to the separation of cells into fractions differing in size and shape and possessing different functions. However, as with the other "non-specific" methods, such as density gradient centrifugation, chromatography on glass beads and nylon column or free flow electrophoresis, these procedures lead at best to the isolation of repertoire of cells enriched with respect to a given type

of cells possessing a distinct immunological function, i.e. hemolysin-forming cells, rosette-forming cells, suppressor cells, null cells, natural killer or cells involved in graft-versus-host reaction. To overcome this problem, specific methods such as affinity chromatography with antigen or antibody chemically coupled to agarose, Sepharose, Sephadex or polyacrylamide gels have been used widely. However, large numbers of cells adhere nonspecifically to the polymeric matrix due to their stickiness and it is difficult to release intact viable cells from the immunosorbents.

The net electrical surface charge of an immunocyte, measured in terms of the corresponding electrophoretic mobility, is the result of charges contributed by the immunoglobulin-like molecules of the antigen binding receptor sites and by the diverse chemical groups constituting the rest of the complex cell membrane. It has been shown in this laboratory (149) that conjugates, consisting of the immunizing hapten coupled to different polyionic carrier macromolecules, can specifically bind to the antigen combining sites of the appropriate immunocytes and thus lead to a marked change in the electrophoretic mobilities of these cells. Hence, it was anticipated that this method may be the basis of the development of an immunologically specific procedure for cell fractionation. Therefore, the objective of this study was to develop a reliable and simple system for cell electrophoresis with a view to establishing surface charge properties of immunocytes and the conditions for altering their electrophoretic mobility.

This study was undertaken with the following specific aims:

(1) to construct a closed, all glass microelectrophoresis chamber for the reproducible measurements of the net electrophoretic mobility (EPM) of immunocytes;

(2) to establish the EPM patterns of lymph node cells of control and immunized mice of strain A/J;

(3) to identify hapten specific binding cells in terms of the alteration of EPM of these immunocytes on interaction with synthetic, polyionic conjugates of haptens (or antigens);

(4) to identify hapten specific binding cells by the immunofluorescence technique;

(5) to isolate hapten specific binding cells by specific immunosorbents;

(6) to identify the θ-positive subpopulation of lymphocytes in terms of the alteration of their electrophoretic pattern on interaction with anti-θ antibodies;

(7) to investigate the effect of neuraminidase on the surface charge properties of lymph node cells;

(8) to establish if the *in vivo* administration of the immunosuppressive drug Cyclophosphamide could affect the surface charge properties of the lymph node cells;

(9) to establish if measurements of EPM of peritoneal macrophages could be used as diagnostic properties in systems involving cellular immunity to tumor antigens or synthetic antigens.

LITERATURE REVIEW

I. CELLS INVOLVED IN IMMUNE RESPONSES

(a) The Immunologically Competent Cells

The immune response manifests itself in two basic forms, namely humoral and cellular immunity. Both of these manifestations are due to small lymphocytes (70), whose precursors originate from the bone marrow (117). The immunologically competent cells (68) consist of a very heterogenous population of cells with regard to their origin (39), migratory pathways (92, 165), life span (51, 166), their functional dependence on the central lymphoid organs (thymic or bursal dependence) (183), their functional capacities (236-239, 244, 245, 254, 258), their ability to form rosettes with red blood cells (240-242, 253, 255), their surface markers, immunoglobulins, and receptors (243, 246-248, 250-252) and their radiosensitivity (249).

The delayed type of hypersensitivity reaction represents the classical 'cell mediated immunity' (CMI) reaction. Skin graft rejections represent another example of CMI, since this type of immunity is easily transferable by lymphoid cells from immune to normal animals. The transfer of spleen and lymph node cells into hosts, which are genetically unable to reject them (163), results in another manifestation of CMI designated as the 'Graft Versus Host' (GVH) reaction, which is due to the attack of the grafted cells against the host's - this immunological reaction is manifested by splenomegaly, hepatomegaly and wasting disease.

Cells that are capable of releasing antibodies into circulation are called antibody-forming cells. The detection of antibody formation at the single cell level can be achieved

by variations (87) of the hemolytic plaque technique introduced by Jerne and Nordin (89). Cell surface immunoglobulins can be detected by the method of immunocytoadherence (99, 197, 19), microdrop assays (123, 7), immunofluorescence technique (250, 259) and electron microscopy for detection of anitbody complexes (45).

With the technique of immunocytoadherence using sensitized spleen cells and SRBC under mild centrifugation, one can separate out clusters or 'rosettes' of cells comprising one nucleated cell surrounded by a group of SRBC. The rosette forming cells comprise a) large and small lymphocytes, b) plasma cells, c) blast cells, and d) macrophages (167). These can be explained by the fact that plasma cells secrete antibodies, macrophages have passively bound antibodies which lead to their rosette formation, while the lymphocytes have limited quantity of membrane bound antibodies (50). The presence of 'receptors' on the surface of the lymphocytes has been demonstrated by their ability to bind to antigen coated column; thus, the cells emerging from such columns have no capacity to produce antibodies on adoptive transfer, while the cells eluted from the column are endowed with this capacity (188). Similarly the removal of rosette forming cell by mild centrifugation results in loss of this capacity (24).

Mitchison (108) formulated the 'receptor hypothesis' which was based on the assumption that antigen sensitive cells have antibody-like receptors on their surface which interact with the immunogen.

There are effector cells of cellular immunity (257) which react with antigen leading directly or indirectly to tissue damage. These effector cells have been detected by the macrophage migration inhibition test (44, 256); GVH (163); including Simonsen-CAM test (21); adoptive transfer of delayed hypersensitivity or graft rejection and in vitro damage to target cells (119). The precursors of normoblasts and myeloblasts, which are colony-forming units, are responsible for the formation of erythroid and myeloid cells in irradiated spleens (178). It is possible to use chromosome analysis of dividing Kupffer cells in regenerating liver to detect the presence of macrophage precursors (83). The bone marrow-derived precursors of thymus and lymph node cells have been shown to be the true self-perpetuating stem cell pool of the lymphoid system by the parabiosis technique (120).

(b) The Thymus and Central Lymphoid Tissue

The functional capacity of the thymus had not been visualized until Miller (112) showed that neonatal thymectomy in mice prevented the proper development of all cell-mediated and some antibody responses. Neonatal thymectomy resulted in impaired ability to reject grafts, to induce GVH reactions, and to make antibody to some antigens especially proteins, bacteria, and red cells. Miller's finding on the importance of thymus in the immune response was in accord with the clinical studies of Good (67), who associated the human congenital immunological deficiency diseases with the independent deficiency of this subpopulation of cells. This phenomenon was also well demonstrated with the aid of chicken as an experimental model (185).

The population of cells that fail to develop after neonatal thymectomy was termed 'thymus-dependent' lymphocytes (T cells). Their blast transformation can be induced by plant mitogens such as phytohaemagglutinin (PHA) and inactivated by heterologous antilymphocyte sera (ALS) (48). They also have the ability to release mediators upon the interaction with specific antigens or mitogen (9, 20, 36, 43, 44, 48, 94, 97, 136, 138, 184, 206).

The study of the T cells in immune responses was made possible with the discovering of their antigenic markers. The existence of a system of isoantigens (Θ) in mice specific for the thymus-dependent cells was described by Reif and Allen (377), using dye exclusion cytotoxicity tests. This antigen was found to be present in brain and in a subpopulation of lymphocytes in the lymph nodes and spleen of the animals. The antigen was found to be controlled by a single locus with two alleles: Θ-AKR was found in AKR, RF and a few substrains while Θ-C_3H was found in most other inbred strains of mice (225). Anti-Θ antisera can be prepared by injecting C3H mice thymocytes into AKR mice or vice versa. Other alloantigenic systems such as TL (thymus leukemia) Ly, and MSLA (mouse specific lymphocyte antigen)were extensively reviewed by Raff (378).

(c) The Bone Marrow and Hemopoiesis

It has been found that x-irradiation of mice with doses between 800 to 1000 rads results in the death of mice within two

weeks unless the animals are injected with hemopoetic cells so as to restore mainly their granulocytes and platelets. With radiomarkers and chromosome markers, it has been shown that the restorative cells are of donor origin (59). Bone marrow is found to be the best for restoration while spleen and peripheral blood are less effective and lymph node, thymus and thoracic duct lymphocytes are ineffective. Colony forming units in the spleen can be observed with the donor cells in limited numbers (7). Each colony is shown to originate from one cell precursor (13), which is found to be related to the small lymphocytes (41).

It is interesting to note that though bone marrow can repopulate spleen, lymph nodes and the thymus, spleen and lymph node cells can only repopulate the spleen and the lymph nodes, but not the thymus (60). Hence the bone marrow can be considered as the ultimate source of the stem cells which are capable of supplying all the other lymphoid organ through the pluripotential precursors whose developments are dependent on the local humoral environments (195). With the thymectomized animals, there is a reduction of the repopulated lymphocytes, hence one of the lymphocyte-inducing environments is provided by the thymus. There has been effort spent trying to identify the non-thymus derived cells, some of the more successful markers being the mouse-specific B lymphocyte antigen (MBLA) as defined by Raff _et al_. (145) and the cell surface immunogloblin by Greaves and others (71, 247, 264).

(d) The Macrophages

Macrophages are found to be responsible for the elimination of foreign material from the circulation and its degradation (37,

207). These cells are also important for concentrating the antigen and presenting them to the immunocompetent cells (107). Studies on animals exposed to total body x-irradiation have shown that the injection of macrophages with antigen promotes the recovery of the immune reponse (52, 140). Antibody formation to SRBC can be induced *in vitro* only in the presence of macrophages (53, 61, 121).

There are also reports, which are disregarded by most modern immunologists, to the effect that the reaction of macrophages with antigen yields an "immunogenic RNA" extract which, when added to lymph node cells in culture or upon injection into animals,may elicit antibody formation (6, 54, 137). Both antigen-induced transformation and antibody responses to some antigens *in vitro* require the participation of macrophages (80, 160).