MEDICINE GRAND ROUNDS

THERAPEUTIC STRATEGIES

IN

CLINICAL IMMUNOLOGY

BY

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JUNE 29, 1978

"The central issues of clinical immunology,...are the production of desired immunity and the elimination of undesired immune reactions."

Robert Schwartz, 1969

1. The scope of clinical immunology

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1. The scope of clinical immunology

With the rapidly increasing application of basic immunology to the diagnosis, prognosis, therapy and prevention of human immunologic diseases there has developed a progressive awareness of the role of clinical immunology in medical practice (1). As a consequence, special committees of the American Association of Immunologists, the British Society for Immunology and the World Health Organization have written papers outlining the aims and functions of immunology as a clinical discipline.

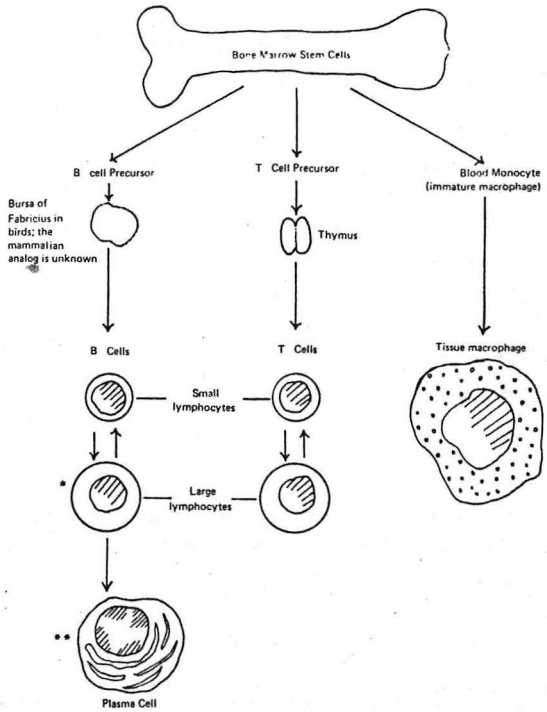
WHO has supported research and training centers for immunology in several parts of the world for a number of years. In fiscal terms immunology has been very successful. Consider the economic benefits of the eradication of poliomyelitis, and of hemolytic disease of the newborn, the development of the measles vaccine and of techniques for detection and prevention of hepatitis B, as just a few examples.

Clinical immunology is important for practically everyone of the organ associated specialties. In the developing countries a major concern is related to work with immune reactions in chronic infectious diseases. This may involve such diverse things as immunodeficiency in patients with lepromatous leprosy, involvement of the complement system in shock due to dengue virus-antibody complexes, or nephrotic syndrome due to immune complexes in malaria. Immunologic investigations have had an important impact in the area of the rheumatic diseases, renal diseases, neurology, transplantation, hematology, allergy, cancer and of course diseases of immunodeficiency.

In all of these areas clinical immunology has focussed on understanding the pathogenesis, improving the diagnosis, developing methods of therapy and monitoring the results of treatment using immunologic techniques.

2. Pathophysiology and pathogenesis

The cellular basis of the immune response is generally understood as the result of interactions between three major types of cells (Fig. 1). Each of them originates from undifferentiated stem cells in the bone marrow, from which develop T lymphocytes, B lymphocytes and macrophages. A genetically directed program of differentiation and generation of diversity, independent of external influences or antigenic stimulation, leads to the development of a variety of distinct T lymphocytes which can be characterized by the presence of cell surface markers and which identify groups of T cells



. Maturation pathways of the principal cells in the immune response. Antibody molecules are secreted by large lymphocytes (*) and especially by plasma cells (**) in the B-cell lineage. Lymphocytes of B and T lineages are morphologically indistinguishable.

Figure 1

with different functions (Table 1). Thus, thymocytes appear to be involved in self recognition, tolerance and the generation of precursors of antigen specific clones. Helper T cells have a positive influence in the course of B cell activation by specific antigens and amplify the response of other T cells. The effector population is largely responsible for the initiation of delayed type hypersensitivity probably through the production and secretion of lymphokines and the interaction with macrophages. Suppressor T cells have an important role in the regulation of the immune response by specific inhibition of either B cell or T cell reactions. Cytotoxic killer T cells develop in the course of the allo-

Table 1

DIVERSITY OF T CELLS (Ref. 2)

Class of T Lymphocytes	TL	Thy-1	Lyt	FcR	Ia	Main Functions
Thymocytes	+	++	1,2,3	-	±	self recognition
T-helper	-	+	1	IgM	-*	B and T cell help
T-effector	-	+	1	?	-	delayed hypersensitivity
T-suppressor	-	+	2,3	IgG	I-J+	B and T cell inhibition
T-killer	-	+	2,3	+	-	cell mediated lysis

* Helper factor has been shown to be a product of the I-A subregion; some specific helper T cells are I-J positive (Tada, T, Takemori, T, Okumura, K et al, 1978)

graft response and are likely to be of importance in the surveillance function against the development of neoplasms. In mouse studies the Lyt antigens are powerful tools for the characterization and study of the functional properties of these T cell populations. In man, helper and suppressor T cells can be identified by the specificity in their Fc receptors for IgM and IgG respectively. The Ia antigens which characterize some of these populations are present also on some of the secreted products that serve as mediators of cell interactions and appear to bear an important relationship to the immune response (Ir) genes of the main histocompatibility complex.

A similar degree of diversity exists among the lineage of B lymphocytes (Table 2). The pre-B cell is a lymphocyte that has small amounts of cytoplasmic IgM and lacks surface immunoglobulin by the usual technique of immunofluorescence staining. It lacks also a variety of other markers that characterize B lymphocytes. At a subsequent stage, early immature B cells acquire surface IgM and Fc receptors. An interesting characteristic of these cells is that they readily become

Table 2

DIVERSITY OF B CELLS (Ref. 3)

B Lymphocytes	cIg	sIgM	sIgD	Fc	Ia	CR	Mls	Lyb5	PC-1
Pre-B cells	±	?	-	-	-	-	-	-	-
Early B cells	-	+	-	+	-	-	-	-	-
T independent	-	+	+	+	+	±	-	-	-
T dependent	-	+	+	+	+	+	+	+	-
Plasma cell	+	-	-	-	-	-	-	-	+

tolerant when exposed to antigen. The more mature B cells can be classified into T independent and T dependent groups, according to whether or not T cell help is required for their activation in response to antigens. These cells may become memory B cells or undergo terminal differentiation into plasma cells. The latter, which are antibody secreting, activate genetic programs which lead to the synthesis of cytoplasmic immunoglobulin and the assembly of an endoplasmic reticulum. At the same time they lose the surface immunoglobulins and most of the other surface markers of B cells and acquire the PC-1 antigen characteristic of plasma cell differentiation.

Macrophages are probably also similarly complex. They originate from rapidly dividing promonocytes in the bone marrow and circulate as monocytes in the peripheral blood. These cells are adherent to glass or plastic surfaces, they are phagocytic and express both Ia antigens and specific monocyte differentiation antigens on their surface. When they enter the tissue spaces, these cells transform into macrophages and adapt to different functional requirements. A subpopulation of macrophages that have Ia determinants appears to be important in antigen processing and presentation, in the immune response.

2a. Immunologic deficiencies

Defects in the development of these cells can give rise to a variety of clinical syndromes depending on which population or subpopulation is affected. Postulated sites of developmental defects involving T or B lymphocytes, giving rise to various immune deficiency states are shown in Figure 2. Thus

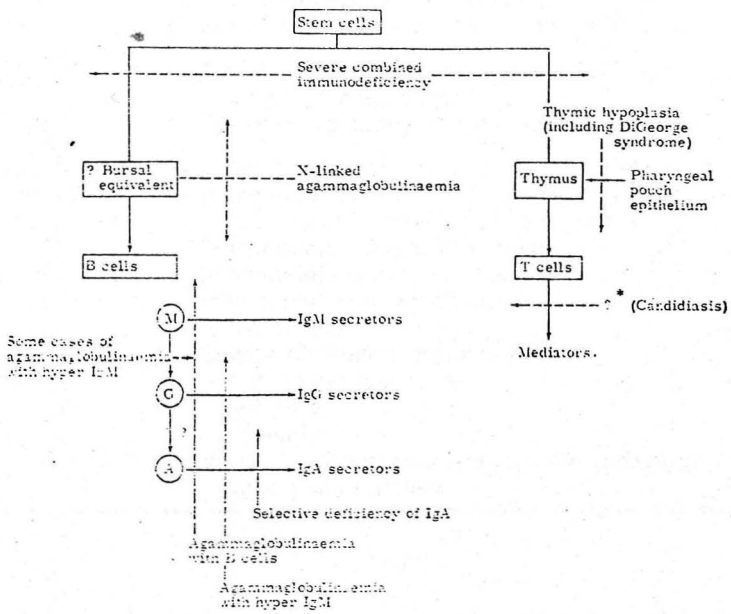


Fig. 1B. Postulated sites of developmental defects (broken arrows) in various immune deficiency diseases.

Figure 2

the lack of stem cell development may result in severe combined immunodeficiency, because precursors for development of both T cells and B cells are absent. Patients with thymic hypoplasia, who are lacking the epithelial portion of the thymus, do not possess the micro-environment required for the differentiation of lymphocytes to mature T cells. In other patients the maturation of B cells is defective. Depending on the site of the defect such patients may be totally lacking in immunoglobulin producing cells, or may have a selective

TABLE 3. Primary Immunodeficiency Disorders*

Probable defect in	Disease
I. B cells	Infantile X-linked aggammaglobulinemia Selective Ig deficiency (usually IgA) Transient hypogammaglobulinemia of infancy X-linked Ig deficiency with hyper-IgM
II. T cells	Thymic hypoplasia (pharyngeal pouch syndrome or DiGeorge's syndrome) Episodic lymphopenia with lymphocytotoxin
III. T cells, B cells, and stem cells	Immunodeficiency with ataxia-telangiectasia† Immunodeficiency with thrombocytopenia and eczema (Wiskott-Aldrich syndrome)‡ Immunodeficiency with thymoma Immunodeficiency with dwarfism Immunodeficiency with generalized hematopoietic hypoplasia Severe combined immunodeficiency Autosomal recessive X-linked Sporadic Variable immunodeficiency (commonest type, largely unclassified)

immunoglobulin deficiency. A more complete classification of the primary immunodeficiency disorders is given in Table 3. Therapeutic strategies involving the transplantation of tissues which are lacking in some of these patients will be discussed below. They constitute remarkable achievements.

2b. Immunologic injury and autoimmunity

Whereas the primary immune deficiencies constitute relatively rare failures of the immunologic system, the concept that immune reactions can be injurious pervades almost every aspect of medicine (4). The basic kinds of immune reactions that may have harmful effects are shown in Figure 3. In Type I reactions, the antigen acts on tissue cells, passively sensitized by antibody, leading to the release of

pharmacologically active substances such as histamine, serotonin and slow reactive substance of anaphylaxis.

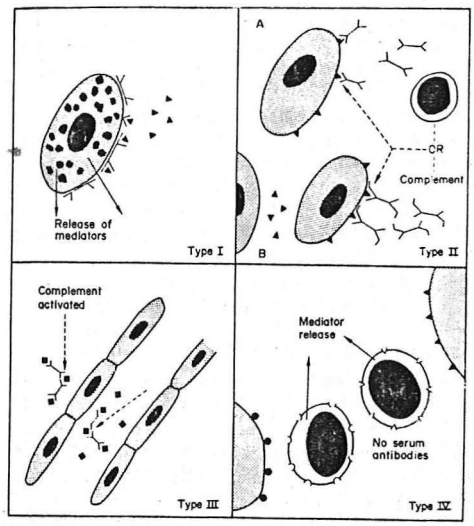


Figure 3

Type II reactions are complement dependent, cytotoxic phenomena in which the antibody reacts with an antigen on the cell surface and lysis occurs due to activation of the complement sequence.

The third type of reaction is caused by the deposition of antigen-antibody complexes. Injury due to these complexes, which may deposit for example in the glomeruli of kidney, results from the inflammatory response they evoke.

In the cell mediated or delayed type hypersensitivity reactions, T lymphocytes accumulate in the tissue, where in response to specific antigen they either have a direct cytotoxic effect or they call forth a chronic inflammatory reaction, through the action of mediators called lymphokines. Attraction, immobilization and activation of macrophages, at the site of the lesion usually constitutes a major mechanism of tissue damage in this type of reaction.

These pathogenetic mechanisms may operate singly or in various combinations. The antigens can be of exogenous or endogenous origin. In addition to the above, another mechanism, which operates preferentially as an autoimmune phenomenon, should be mentioned. It consists of production of antibodies against specific receptors. Anti-receptor antibodies may have a stimulating effect as in the case of Graves' disease, or a blocking effect as in the case of pernicious anemia or myasthenia gravis.

Myasthenia gravis constitutes an interesting example of disease caused by an autoimmune reaction (5). Myasthenia gravis is a neuromuscular disorder manifested by weakness and fatigability of voluntary muscles. Because of the similarity between myasthenia gravis and curare poisoning and the remarkable response to anticholinesterase drugs it was known for years that the abnormality resided in the neuromuscular junction. The frequent presence of abnormalities in the thymus and of various kinds of auto-antibodies in the serum, suggested years ago that an immune mechanism might be operative.

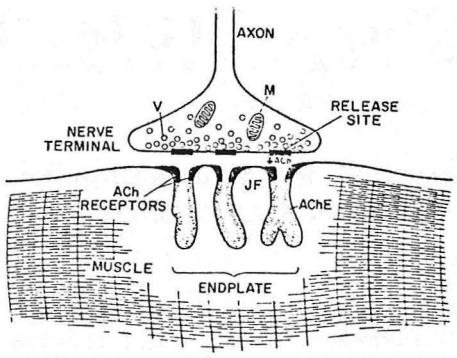


Figure 4

However, the exact nature of the process remained elusive. Only during the past 4 years has it become clear that the basic defect in this disease is a reduction of available acetylcholine receptors at the neuromuscular junctions brought about by an autoimmune attack. The majority of patients with myasthenia gravis have antibodies against the acetylcholine receptors. Injection of patient's serum into mice was shown to reproduce the syndrome of weakness resembling myasthenia gravis. Patients with neonatal myasthenia, born of mothers with myasthenia gravis, were found to have acetylcholine receptor antibodies which persisted for about 2 weeks and correlated with the clinical findings. By immunization of

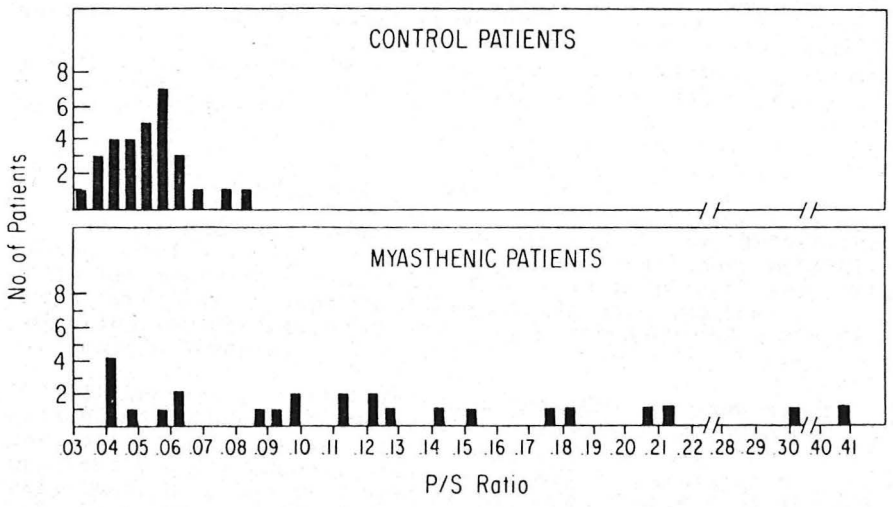


Figure 5 Acetylcholine Receptor Antibodies in Myasthenic Patients and Controls.

animals with purified acetylcholine receptors it is possible to produce a disease which closely resembles human myasthenia gravis. As in the human disease, the animals show typical electromyographic changes, a decrease of available acetylcholine receptor sites and ultrastructural changes similar of those of the human disease. Lymphocytes isolated from patients with

myasthenia gravis synthesize antibody to acetylcholine receptors *in vitro* and there is some evidence that a cell mediated immune response against acetylcholine receptor antigen may exist also, in addition to the production of humoral antibodies. Most of the evidence however suggests that the antibody is largely responsible for the clinical lesion. In one set of experiments it appears that the antibody by itself can accelerate acetylcholine receptor degradation. Other experiments suggest that the complement system may be involved.

Another disease in which a circulating auto-antibody appears to play an important role, is Goodpasture's syndrome and glomerulonephritis due to anti-basement membrane antibody. Experimentally this is a lesion that can be reproduced either by active immunization with glomerular basement membrane antigen or by passive administration of antibody. The complement sequence is involved in the development of the tissue injury (6). Both of these conditions may sometimes be treated by removal of the harmful antibody. This will be discussed below in section 3b.

2c. Defective immune regulation

The immunologic network is a balanced system through the operation of a variety of stimulating and inhibitory mechanisms. It is becoming increasingly clear that inhibitory or suppressor mechanisms play an important homeostatic role and that defective immune regulation may lie at the heart of a number of common disorders.

It appears that the efficient operation of suppressor mechanisms is needed to prevent the production of auto-antibodies against a variety of antigens. B cells are apparently quite capable, if appropriately stimulated by polyclonal B cell activators, to produce a variety of auto-antibodies (7). Injection of lypopolysacharide into experimental animals has resulted in the production of anti-DNA antibodies, rheumatoid factor-like antibodies reacting with IgG, auto-antibodies to red blood cells and possibly even antibodies against some histocompatibility antigens. Self-reactive B lymphocytes have also been found in humans. B cells from normal donors were found to be capable of producing autologous cytotoxic antibodies after stimulation by lypopolysacharide and rheumatoid factor after stimulation with Epstein-Barr virus. Certain patients who develop auto-antibodies, may be deficient in a population of suppressor T lymphocytes as has been recently shown by Bresnihan and co-workers (8) in patients with systemic lupus erythematosus.

On the other hand, excessive immune suppression may lead to severe immune defects. Certain patients with hypogammaglobulinemia have been found to have an excess of suppressor T cells (9). Excessive suppression may also underlie the defective delayed hypersensitivity in patients with Hodgkin's disease and sarcoidosis (9).

Laboratory tests are now being developed for the routine diagnosis of these defects. Specific modes of therapy will soon be available that will allow the correction and manipulation of defective homeostatic mechanisms, followed by sequential monitoring of lymphocyte functions to determine the long term re-establishment of an operational balance.

3. Therapy

This discussion shall be limited to selected topics chosen to illustrate the application of basic immunology to the treatment of specific clinical problems, and therapeutic strategies that are relatively new. Thus, the section on transplantation will be limited to transplants performed for the reconstitution of patients with various types of immunodeficiencies. The transplantation of bone marrow for treatment of malignancies or marrow aplasia, and the transplantation of organs such as the kidney, liver, or heart will not be discussed. Much of the forthcoming discussion will be devoted to therapeutic strategies for the treatment of patients with disease produced by immunologic mechanisms, autoimmunity, or defective immune regulation. Recently developed procedures or drugs will be discussed, fully realizing that it takes years to prove their efficacy. The natural history of these diseases is variable and there are frequent spontaneous remissions. It takes a long time to organize and evaluate the necessary controlled trials. These forms of therapy also find application in the treatment of patients with neoplasms, but it would not be possible to do justice to all the interesting developments in cancer immunotherapy, in the available space.

3a. Transplantation

Bone marrow transplantation has been used successfully in children with immunodeficiency. The objective is to supply a source of stem cells that can differentiate into immunologically competent T and B lymphocytes (10). Because of the relatively weak immune reactivity of the recipients, intensive immunosuppression is not needed to obtain bone marrow engraftment. Still in many cases only partial engraftment is achieved and

repeated attempts are necessary. Recently, successful marrow engraftment was obtained in patients with the Wiscott-Aldrich syndrome after total body irradiation. It was speculated that room had to be made for the engraftment of the transplanted bone marrow cells.

The main problem in this type of bone marrow transplantation is the development of graft-vs-host disease. HLA matching can reduce the frequency and severity of this complication. Methotrexate and various other drugs are commonly employed for prevention or treatment of graft-vs-host disease manifestations. Genotypically HLA identical sibs are most commonly used with success. The HLA antigens constitute the strongest immunogenic and target antigens in both host-vs-graft and graft-vs-host reactions. The mixed lymphocyte culture stimulating antigens, coded by the HLA-D region, have been found to be particularly important because of their predominant role in graft-vs-host disease. Since HLA identical siblings are often not available, the possibility of using mixed lymphocyte culture matched but otherwise mismatched related or even unrelated donors is of considerable interest. Results in 8 such recipients are shown in Table 4

Table 4

Lymphoid Reconstruction of SCID Following Transplantation of Marrow From Donors Other Than Genotypically Identical Siblings

Patient	Donor	Degree of HLA Compatibility	HLA Incompatibilities	Engraftment of Donor Cells	GVHD	T-Cell Reconstruction	B-Cell Reconstruction
1	Uncle	Genotypic	None	T cells	None	+ (donor)	+ (host)
2	Father	Genotypic	None	T cells	Mild	+	–
3	Father	Phenotypic	None	Full	Severe	+	+ (delayed)
4	Father	Phenotypic	None	Full	Mod	+	+
5	Uncle	Nonidentical	B7, B12	T cells	Mod	+	–
6	Sibling	Nonidentical	Bw37	Full	Mod	+	+
7	Mother	Nonidentical	A2	Full	Severe	+	+
8	Father	Nonidentical	A2	Full	None	+	+

(O'Reilly et al, 1978). Recently developed methods for HLA-D region typing is making it easier to select donors from large panels of HLA-D typed subjects.

Not all patients with severe combined immunodeficiency respond well to bone marrow transplantation. Some of these patients have improved after a subsequent thymus transplant. On the basis of *in vitro* tests of the capacity of lymphoid precursors to differentiate O'Reilly and co-workers (1978)

have suggested that there may be at least 3 types of differentiation abnormalities that may contribute to the pathogenesis of severe combined immunodeficiency. In the classical form, stem cells capable of differentiating in the presence of thymic epithelium or thymic factors are absent. In other patients, precursors are present which can be induced *in vitro* by thymic factors to express certain T cell characteristics. However they were defective in that they failed to develop into sheep RBC rosette forming cells or to acquire the functional activities of T lymphocytes. Finally, in other patients with SCID, differentiation of functional T lymphocytes is observed following culture of bone marrow cells with normal thymic epithelium. In such cases the abnormal component may be the patient's thymus.

In such patients as well as in other patients in whom the primary defect is a failure in the development of the epithelial portion of the thymus, transplantation of an allogeneic fetal thymus has been apparently quite successful in restoring immune function (11). This would appear to be a physiologic approach, the success of which may depend on the immaturity of both the host and the transplanted tissue permitting the establishment of a mutually tolerant state. Recent experiments by Zinkernagel (12) dealing with the process of development of self-recognition in the thymus, have suggested that there may be problems with the use of allogeneic thymus tissue that have not been previously recognized. On the basis of experimental work with mice, it has been suggested that patients reconstituted with HLA-A, B and D incompatible thymus allograft may remain immuno-incompetent, except for allograft rejection, and mitogen effects, for which syngeneic self-interaction of lymphocytes is not necessary. The mouse experiments suggest that self-interaction is required for the normal development of immune responses to most antigens. This self-recognition appears to depend on an interaction between lymphoid precursors and epithelial cells in the thymus. Determinants of the same type as presented in the thymus are recognized on the surface of macrophages presenting antigen to the T cells. According to this model, if the thymus cells and the macrophages don't match for HLA, the immune response may be impaired.

3b. Removal of lymphocytes or plasma

The removal of lymphocytes by placing a fistula in the thoracic duct and draining the flow of lymph, has been used extensively in experimental animals. It produces a progressive depletion of lymphocytes from the peripheral circulation and if maintained, results in a marked decrease

of lymphoid cells in the peripheral lymphoid tissues. The procedure has been used to study the functional activities of the recirculating lymphocyte pool and has been found to produce a profound state of immunodepression. In recent years lymphocyte drainage has been used as a means of producing immunosuppression in humans. It has been used successfully as the main form of immunosuppressive treatment in patients undergoing clinical kidney transplantation (13). It is of course not practical for long term maintenance and it is too cumbersome a procedure to gain widespread application.

Thoracic drainage as immunosuppressive treatment for a variety of auto-immune diseases is an experimental procedure of considerable interest, but thus far of little practical use (13).

Pearson and co-workers (1975) have treated a number of patients with severe rheumatoid arthritis. As can be seen in Table 5, their patients had high titers of rheumatoid factor and severe manifestations of systemic rheumatoid disease.

Table 5

PATIENT CHARACTERISTICS (NINE WOMEN WITH RHEUMATOID ARTHRITIS)

Patient	Age	Rheumatoid Factor	Nodules	Antinuclear Antibodies	Days of Drainage	Total Number of Cells Drained	Comments
I.P.	24	1:2560	yes	1:64	46	17×10^{10}	disease began age 16
M.B.	47	1:10,240	yes	1:64	19	27×10^{10}	history of cerebral vasculitis (?)
J.R.	55	1:10,240	yes	1:256	81	36×10^{10}	—
D.M.	50	1:2560	yes	0	30	41×10^{10}	febrile episodes
D.S.	65	1:5120	no	+ undil.	26	19×10^{10}	splenectomy for Felyt's syndrome
G.P.	38	1:10,240	yes	1:16	82	106×10^{10}	cyclophosphamide and azathioprine failure
J.G.	38	1:1280	yes	1:16	105	111×10^{10}	second attempt at drainage
A.S.	53	1:2560	yes	equiv.	19	29.8×10^{10}	leukopenia
C.W.	37	1:5120	yes	1:4	73	28.2×10^{10}	azathioprine, cyclophosphamide, chlorambucil failure

Two of them had failed to respond to immunosuppressive drugs, including cyclophosphamide, azathioprine and chlorambucil. Drainage of lymphocytes was maintained from 19 to 105 days.

Immune response testing showed loss of delayed hypersensitivity skin test reactions, with little effect on the production of humoral antibodies. The study was not controlled in any way, but the clinical response was quite impressive. Subjective improvement began after about 7 days and was maximal 10 to 14 days after initiation of thoracic duct drainage. Sinovial effusions were diminished, the number of painful joints decreased, rheumatoid nodules decreased in size and grip strength improved (Fig. 6). Thoracic duct drainage treatment has also been used successfully in systemic lupus erythematosus with cutaneous vasculitis and in myasthenia gravis.

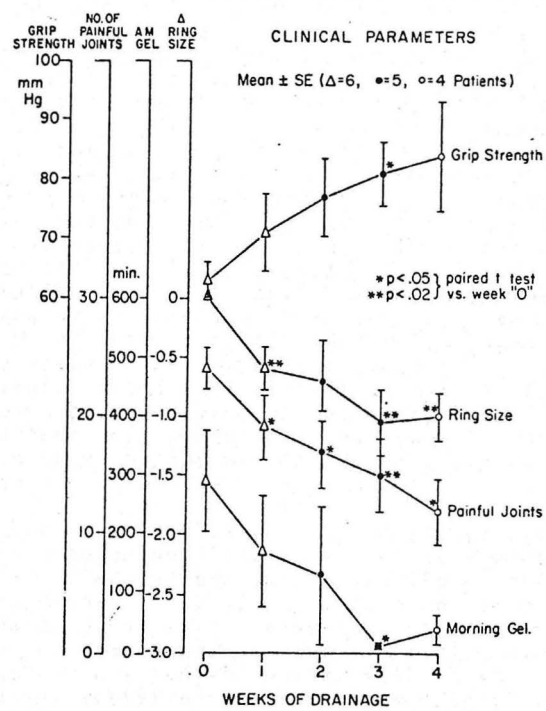


FIGURE 6. Measurement of various clinical parameters as indicated in several patients who experienced lymphocyte depletion via thoracic duct drainage over a 4-week period. Note that grip strength (a measurement of improvement) increased, whereas ring size, painful joints, and the degree of morning gel progressively diminished as lymphocyte depletion was under way. Statistical significance of results is as indicated.

Removal of plasma with the objective of reducing the levels of autoantibodies or immune complexes is another

relatively new mode of therapy. Of course plasmapheresis is commonly used as a method of obtaining component blood products. The procedure can be done by hand or when larger volumes are exchanged by continuous flow blood cell separation. Up to 4 liters can be exchanged in an adult and replaced with either fresh frozen plasma or purified protein fraction, which is mainly albumin. Intensive plasma exchange is required to remove harmful macromolecules. As a result, plasma proteins such as immunoglobulins, complement components, and clotting factors are also depleted.

In the treatment of Goodpasture's syndrome, plasmapheresis was combined with immunosuppressive drugs. The aim was an immediate removal of antibodies by intensive plasma exchange and long term control of anti-glomerular basement membrane antibody synthesis by immunosuppressive drugs. Seven patients were treated by Lockwood and co-workers (14). Four of them were anuric at the beginning of treatment and although antibody to GBM was somewhat reduced, return of renal function did not occur. The other 3 patients showed considerable improvement. The therapeutic regimen appeared to control pulmonary hemorrhage in all 5 patients suffering from this complication. The relative contribution of steroids, cytotoxic drugs and plasma exchange to the beneficial results was difficult to evaluate. The observation that withdrawal of immunosuppressive drugs was followed by an immediate, progressive rise in the titer of anti-GBM antibody, lead the authors to believe that the immunosuppressive drugs made an important contribution to the treatment. Withdrawal of these drugs was followed in several cases by recurrence of pulmonary hemorrhage. It was emphasized that improvement of renal function occured in patients in whom therapy was initiated early in the course of the disease.

A number of investigators have utilized plasmapheresis combined with immunosuppression in the treatment of myasthenia gravis (14). Pinching and co-workers (1976) treated 3 patients with severe disease, all of whom improved. Dau and co-workers (1977) treated 8 patients. Plasmapheresis was combined with prednisone and azathioprine. The initiation of plasmapheresis was followed by a rapid and marked fall in the level of antibodies against acetylcholine receptors (Figure 7). At the same time the patients were reported to show a significant increase in strength (Table 6). Obviously plasmapheresis would not be utilized in patients with mild myasthenia gravis. But in patients with myasthenic crisis, or who are deteriorating rapidly, in patients with respiratory difficulties, and progressive disability in spite of optimal therapy with anticholinesterase drugs and steriods, or in patients with respiratory impairment and pulmonary infection, in

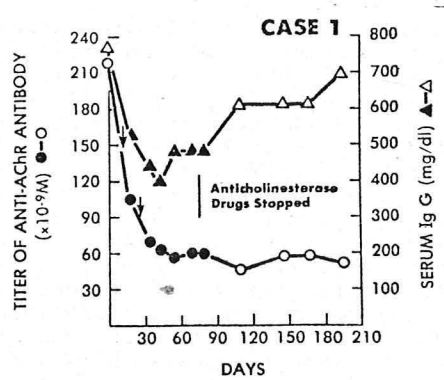


Figure 7 Serial Determinations of Titer of Antibody to Acetylcholine Receptor (Anti-AChR) and Serum IgG

whom immunosuppression might be contraindicated, there may well be a place for the use of plasma exchange.

Jones and co-workers (1976) reported the use of plasma exchange in the treatment of 8 patients with systemic lupus erythematosus. Four of the patients had circulating immune complexes and were said to be improved after the treatment. In the 4 patients in whom complexes were not detectable, improvement was not observed.

This is obviously a highly experimental therapeutic procedure. The reports are very preliminary. The procedure puts a strain on the blood bank, especially if a continuous flow separator is not available. In the future, if controled studies confirm the preliminary reports, this somewhat complicated therapeutic procedure may have certain indications in patients who do not respond to other methods of treatment.

Table 6. Clinical Features of Patients with Myasthenia Gravis.

Case No.	Sex	Age	Clinical Class*	Duration of Myasthenia Gravis	Interval since Thymectomy	Thymic Lesion	Duration of Prednisone Therapy	Response to Plasmapheresis*
		yr		*yr*	*yr*		*yr*	
1	F	56	III	3.2	2.8	Invasive thymoma	2.5	A
2	F	29	III	2.5	2.3	Hyperplasia	2.3	B
3	F	51	IV	7	1.3	Atrophy	5	C
4	F	53	III	5	5	Invasive thymoma	0.3	B
5	M	34	IV	15	13	Hyperplasia	5	B

*According to Osserman[24] (A denotes complete remission, and C moderate improvement).

3c. Immunosuppression

The use of pharmacologic agents to inhibit undesired immune reactions is a logical approach that has gained wide acceptance in the practice of medicine. Thousands of kidney transplants are successfully performed with the aid of drugs

that eliminate or decrease rejection. Use of these drugs is also common in a variety of patients with immunological disorders. In considering the use of these substances the physician must put in the balance the many toxic side effects. The dangers include various types of acute toxicity, the impairment of defenses against infections, and the long-term danger of neoplasia. Other important factors, that should be taken into account, are the accuracy of the diagnosis and the life threatening nature of the disease, as well as the potential for recovery. Evaluation of the effectivness of immunosuppressive agents in autoimmune diseases is complicated by the heterogeneity of patient groups and the unpredictable clinical course. It is clear that only properly controled trials in which patients are assigned randomly to various treatments can show which drugs are of use in which dosage, and in which conditions. Fortunately, many such trials have been conducted during the last few years and useful information is now available for the treatment of many conditions (15).

Steroids are probably the most widely used immunosuppressive agents. They are also strong anti-inflammatory drugs. However, it was recently found that a single dose of methylprednisone given intravenously to human volunteers made their B cells hyporesponsive to T cell help. Less immunoglobulin was produced and at the same time decreased the T cell suppressor activity was observed. T cell helper function remained unchanged (16). The net effect in normal individuals was a reduction of immunoglobulin synthesis. It was suggested that the effect on suppressor T cells might explain the improvement of delayed hypersensitivity after steroids in patients with sarcoidosis and the appearance of immunoglobulin in common variable hypogammaglobulinemia with suppressor cells. On the contrary, in lupus, where suppressor activity is diminished, the inhibitory effect on B cells might be magnified.

Azathioprine, cyclophosphamide and chlorambucil are widely used for immunosuppression and have been carefully studied in a number of conditions. Patients with severe rheumatoid arthritis, who were unresponsive to other forms of therapy, have been shown in several controlled trials to respond to these drugs. Of particular interest was the study with cyclophosphamide performed by the Cooperating Clinics of the American Rheumatism Association.(17).

The following case history is that of a patient with severe rheumatoid arthritis, who was treated with alkylating agents and went into complete remission which has lasted over 2 years.

* * *

C.R. PMH # 282837. This black woman, born June 4, 1926, developed joint pain and swelling in 1961. The arthritis involved the small joints of her hands and subsequently the shoulders, hips, knees, and wrists. In 1963 she was bedridden for 9 months because of severe arthritis. When first seen in October 1969, both wrists were tender with swollen synovium. MCP and PiP joints were swollen and tender, there were effusions in both knees, the ankles were swollen, tender, and warm, the toes and feet were also involved. She had episcleritis. The erythrocyte sedimentation rate was 51mm/per hour, the latex test 3+, the sheep cell agglutination titer was 1:448. She was treated with aspirin and anti-inflammatory drugs, including prednisone up to 10 mg per day and received a full course of gold treatment with no benefit. Because of the severity of the arthritis and the worsening of the episcleritis she was started on Cytoxan in March of 1971. She took Cytoxan 150 mg per day and prednisone 15 mg per day until September 1973. Her arthritis continued to be active. At this time chlorambucil was started instead of cyclophosphamide. The dose varied between 6 and 12 mg per day. In April 1975, she was found to be improved. In September, still on chlorambucil, she was asymptomatic. In March 1976, she missed her clinic appointments and stopped taking her medications. She was in complete remission and was given only aspirin and 4 mg of prednisolone a day. She was last seen May 16, 1978, still in complete remission more than 2 years after the immunosuppressive drug had been stopped.

* * *

The use of immunosuppressive agents in lupus, particularly lupus with nephritis, has been more difficult to evaluate because of the variability in this disease (18). The beneficial effect of cyclophosphamide on the nephritis of mice with lupus, has been repeatedly confirmed (19). A number of controlled studies now leave little doubt that both azathioprine and cyclophosphamide improve the glomerular lesions of lupus nephritis (20). The study by Steinberg and co-workers (1972) suggested that cyclophosphamide was superior to azathioprine in human lupus nephritis.

The following clinical case is an example of a patient with systemic lupus erythematosus with nephritis, treated first with cyclophosphamide, later with chlorambucil, and now in prolonged remission.

* * *

G.W. PMH #209313. This young black woman has been

treated here since 1965, when at the age of 15 she developed arthritis, a malar rash, anemia and convulsions. The ANA and LE tests were positive. A kidney biopsy showed proliferative glomerulonephritis. An organic psychosis developed and she was hospitalized for 5 months. During her subsequent course she developed polymyositis and steroid induced diabetes requiring insulin. Urinary protein was about 5 gm/24 hrs. Treatment consisted of prednisolone 40 mg and cyclophosphamide 2 mg/kg. While on cyclophosphamide during the ensuing two years, the patient improved and progressively went into complete remission. Prednisolone was reduced to 10 mg and insulin was no longer needed. Cyclophosphamide was given until November 1969, when she developed herpes zoster. Prednisolone was kept at 5 mg, until May 1973, and then, this too, was discontinued. In November 1973, she developed alopecia followed by ankle edema and proteinuria. A repeat kidney biopsy was unsuccessful. Treatment consisted of prednisolone 30 mg and chlorambucil 10 mg daily. The latter was discontinued in early 1975 because of leukopenia. In early 1976, protein in the urine was 300 mg/24 hr., creatinine clearance 106 ml/min. Except for hypertension, controlled with Esidrix, the patient is in remission, leading an active, normal life. She has occasional joint pains. Recently her ANA was positive, anti-DNA (Crithidia) positive undiluted, anti-ENA positive 1/320 not changed by RNAse, CH50 320 units. She is taking prednisolone 12 mg/day and continues to be more or less asymptomatic.

* * *

Cyclophosphamide has been successful in preventing relapse of the nephrotic syndrome in children (21). In the study by Barratt and Soothill (1970) relapses occured in 11 out of 15 patients treated with steroids only, whereas among the 15 patients treated with cyclophosphamide, only 3 relapsed. The drug also appeared to be beneficial to a number of patients who were resistant to treatment with steroids alone.

3d. Immunostimulation

Levimasole is a synthetic antihelminthic drug which appears to have immunostimulating properties, both *in vivo* and *in vitro*. Its mode of action is incompletely understood. There is evidence in murine systems that levimasole can increase the phagocytosis of heterogolous cells by macrophages *in vitro* and accelerate the clearance of carbon particles. Additionally, levimasole may stimulate

delayed hypersensitivity. It was found to increase the graft-vs-host reaction and induce regression of tumors. In man, levimasole has been reported to increase specific delayed hypersensitivity reactions *in vivo*, to enhance the response of lymphocytes to antigen *in vitro*, to increase the number of sheep RBC rosette forming cells, and to increase the activity of the Fc receptors in human monocytes (22).

Levimasole has now been used in the treatment of several human diseases. Preliminary reports of beneficial effects in patients with rheumatoid arthritis have been rapidly confirmed (23). Runge and co-workers (1977) published a small double blind study which showed significant improvement in 14 treated patients, compared to 13 controls receiving placebo. In Figure 8 are shown the current results of the Eular Multicenter trial, which also is a double blind study. Levimasole treated patients showed considerable improvement in the number of tender and swollen joints, in the duration of morning stiffness and in the erythrocyte sedimentation rate. In this European study,

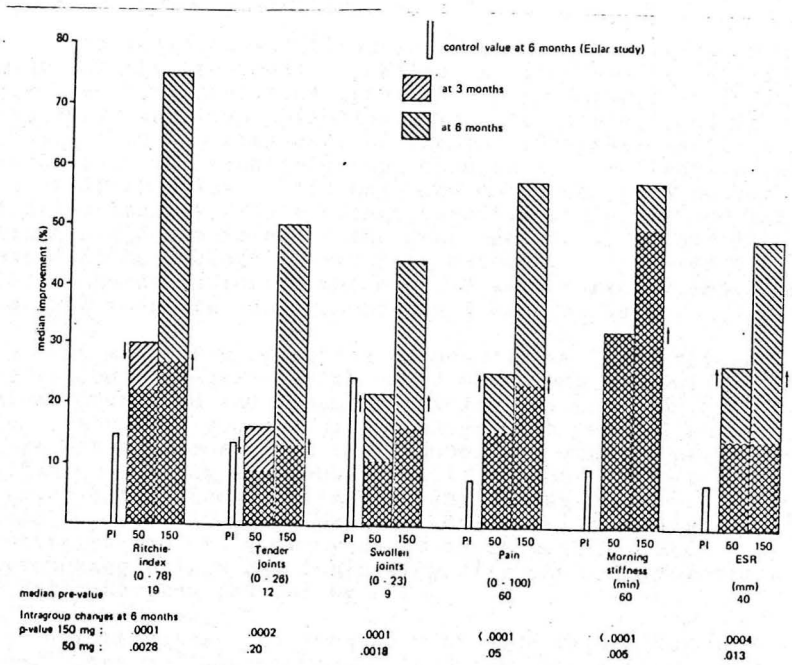


Figure 8

frequent side effects were a skin rash, and a flu-like illness. Leukopenia and agranulocytosis were occasionally observed. Leukopenia occurs after ingestion of the drug. It has been recommended therefore, that the white cell count be checked several hours after each dose is given (24).

Reports on the use of levimasole in systemic lupus erythematosus are very preliminary, but encouraging. The drug has also been used with some success in murine lupus erythematosus, where it has recently been reported to maintain a cyclophosphamide induced remission (25). Other diseases in which levimasole has been tried include ankylosing spondylitis, Crohn's disease, and multiple sclerosis (26). The latter two were unsuccessful. Two out of 8 patients with Crohn's disease developed a severe arthritis which subsided after withdrawal of the drug. Levimasole had an apparent adverse effect on patients with multiple sclerosis. The investigators decided to prematurely terminate the trial.

3e. Other forms of therapy

Penicillamine was first introduced into clinical medicine for the treatment for Wilson's disease. It has since been used as a chelating agent in other diseases including cystinuria and lead poisoning. An early study found it to be useful in the treatment of rheumatoid arthritis (27). The British multicentre group found it to be effective in a controlled trial. This has been confirmed in a number of other studies. In a recent Canadian study, 25% of the patients did not tolerate the drug because of mucocutaneous, renal and hematologic toxic side effects. Of the 17 patients who did tolerate the drug, 8 were markedly improved, 6 showed moderate improvement and 3 had no change.

The mechanism of action of penicillamine is not well understood. It has been observed that immunoglobulin levels decreased and immune complexes disappeared in patients who were successfully treated with penicillamine. In recent experiments on the immunologic effects of penicillamine (28) it was found that it did not inhibit the development of hemagglutinating antibodies *in vivo*. *In vitro* however, it had a profound effect, inhibiting the transformation of lymphocytes by concanavalin A and phytohemagglutinin and inhibiting also the transformation of B lymphocytes induced by LPS.

Unfortunately the frequency of side effects is quite high. They include leukopenia and trombocytopenia, bone marrow aplasia, immune complex renal disease with membraneous glomerulonephritis and nephrotic syndrome, skin

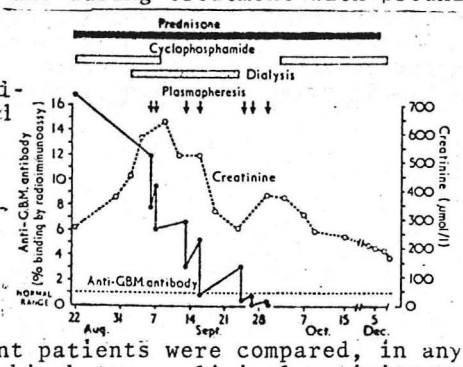
rashes, fever, oral ulcers, and blunting or alteration of taste perception. In addition a group of manifestations that resemble a variety of autoimmune diseases such as Goodpasture's syndrome, myasthenia gravis, polymyositis and a lupus-like disease have developed in patients treated with penicillamine (29).

4. Immunologic testing

Immunologic testing is being used increasingly for follow-up with the purpose of evaluating the results of treatment and for the early detection of exacerbations before clinical changes become detectable. The immunologic monitoring of kidney transplant recipients has become a very active field. The methods used for such immunologic testing have been the object of a number of symposia and publications (30).

4a. Testing for antibodies

The role of several types of autoantibodies in the development of immunologic disease has been discussed above. In Figure 9, is shown the course of a patient with nephritis due to anti-glomerular basement membrane antibody, before and during treatment with prednisone, cyclophosphamide and plasmapheresis.



The anti-GBM antibodies were measured using a radioimmunoassay in which the antigen is a collagenase digest of human glomerular basement membranes which are labeled with radioactive iodine. Although there was no correlation between the binding capacity of the serum and the severity of clinical disease, when different patients were compared, in any given individual, the relationship between clinical activity and serum antibody levels followed over a period of time, appeared to be quite good.

4b. Detection of immune complexes

Not all immune complexes are damaging since sensitive methods of detection have shown low levels in people with no

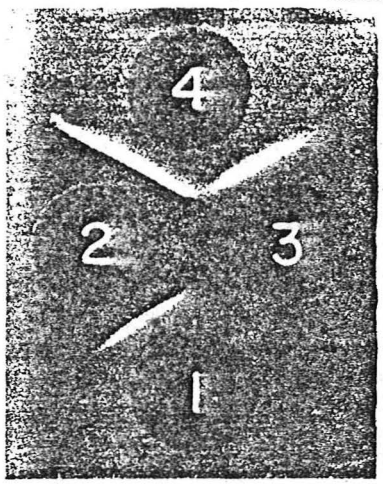
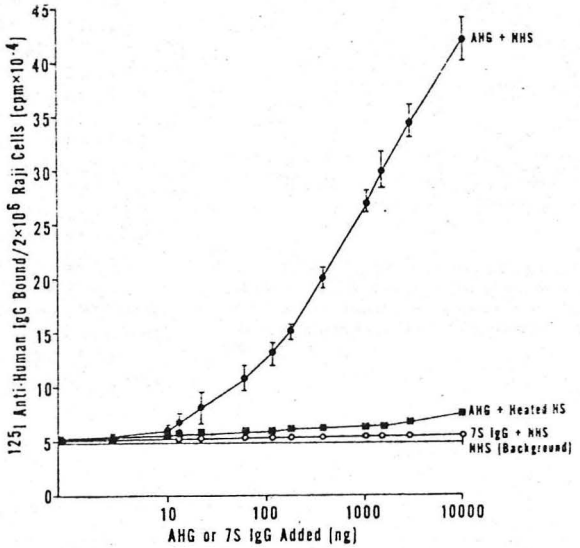
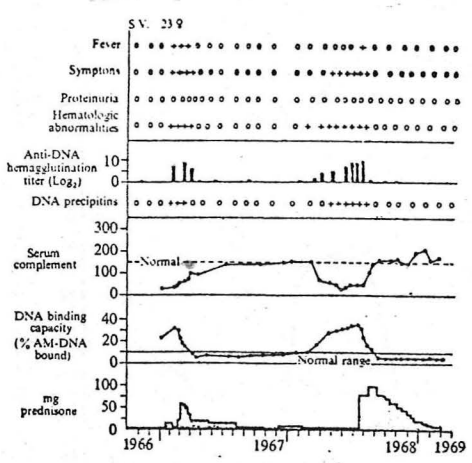


FIG. 10 *Reactions of C1q and 19S rheumatoid factors with aggregated Ig. The effect of reduction and alkylation on the reaction of aggregated Ig and C1q is shown. (1) Purified C1q; (2) aggregated Ig; (3) aggregated Ig reduced and alkylated; (4) purified rheumatoid factor. Reprinted from Agnello et al. (4) with permission.*

apparent disease. Immune complexes vary in size, antibody class, and complement binding capacity, as well as the specific antigen involved. There are many methods for detection of immune complexes none of which is entirely satisfactory. Different methods appear to detect different kinds of complexes. Most of the available techniques depend on physical chemical properties of complexes such as cryoprecipitation or precipitation by polyethylene glycol, on recognition proteins such as human C1q or rheumatoid factor, or on binding to cells which detect complexes by their C3 or Fc receptors (31). A simple method of precipitation of complexes in agar by C1q and purified rheumatoid factor, is shown in Figure 10. In the Raji assay, when these cells are incubated with sera, native 7s IgG is bound through the Fc receptors to a low level, but complexed IgG with fixed complement is bound much more by the complement receptors. When serum containing immune complexes is used, the results are expressed as micrograms of aggregated human gammagobulin equivalent, per ml of serum (Fig. 11).



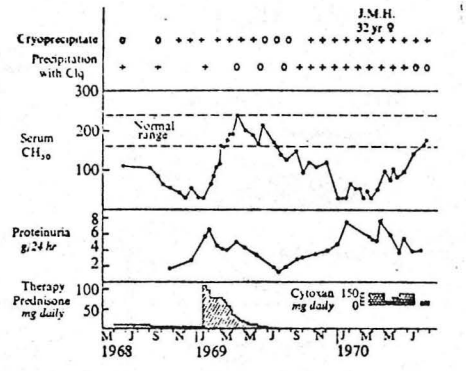
Raji cell uptake of ^{125}I-labeled antihuman IgG after incubation with increasing



Serial study of patient S. V. showing two periods of clinical exacerbations associated with increases in titer of anti-NDNA antibodies and serum complement depression. **Antibodies were assayed by agar gel precipitation, hemagglutination and ammonium sulfate precipitation test using labeled DNA [36].**

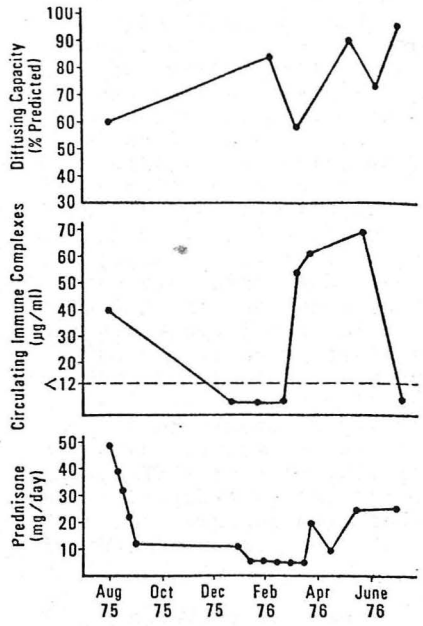
Figure 12

Using one or more of these techniques immune complexes have been found in the circulation of patients with systemic lupus erythematosus, infective endocarditis, ulcerative colitis, Crohn's disease, membranoproliferative and other types of glomerulonephritis, and more recently also in idiopathic intersticial pneumonia and in steroid responsive nephrotic syndrome (32). In Figures 12 and 13, are shown the evolution of two patients with systemic lupus erythematosus. The presence of immunologic factors in the circulation correlated with the clinical course. In Figure 14, are shown the results of studies with the Raji assay in a patient with intersticial pneumonia. The level of immune complexes decreased after steroid treatment and showed a relationship with the difusing capacity of the lung. In patients with steroid responsive nephrotic syndrome immune complexes were detected by inhibition of agglutnation of IgG coated latex particles by rabbit IgM anti-IgG. Interestingly these complexes did not bind Clq. Sequential studies showed a correlation between the level of IgG complexes and the clinical course as shown



Serial study of an exceptional SLE patient (J. M. H.) showing two broad periods of clinical activity with renal disease which were associated with the presence of cryoprecipitins and Clq reactivity in the serum. **No antibodies to polynucleotides were present.**

Figure 13



Relation of Diffusing Capacity with Circulating Immune Complex Level in a Man with Usual Interstitial Pneumonia in Response to Varying Doses of Prednisone.

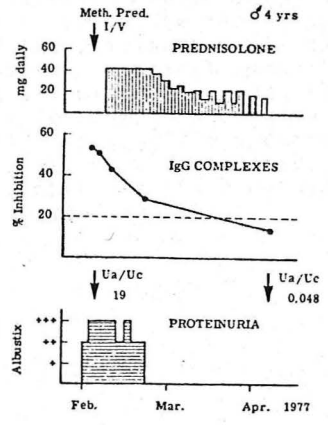
Figure 14

4c. Monitoring the functions of lymphocytes

A few years ago the study of human lymphocytes *in vitro* consisted of performing a cell count, perhaps a differential count of small, medium, and large lymphocytes and a test of lymphocyte transformation with PHA or concanavalin A. More recently with the advances in knowledge of surface markers it became possible to determine the number of T cells by their ability to form sheep cell

in Figure 15.

Figure 15



Sequential Detection of IgG Complexes in a Four-Year-Old Child with Steroid-Responsive Nephrotic Syndrome during Relapse and Remission (Ua/Uc Denotes Urinary Albumin/Creatinine Concentration).

rosettes and the number of B cells by staining for surface immunoglobulins and the presence of complement receptors. During the last 2 or 3 years a variety of functional assays have become available. It is now possible to determine whether the bone marrow stem cells can differentiate into mature T cells under the influence of thymic factors. It is possible to assess separately the functional activity of helper, suppressor, killer, and effector T lymphocytes. The ability of B cells to produce and secrete immunoglobulin with and without T cell help, and with and without participation of macrophages can also be investigated. It is possible to establish a functional profile of various lymphocyte subpopulations in interaction with each other or in combination with cells from normal donors and under the effect of both nonspecific and antigen-specific stimuli. For example in kidney transplant recipients it is possible to investigate the reactivity of different lymphocyte subpopulations of the recipient against the specific transplantation antigens of the donor. With the use of cryogenically preserved donor antigens it is possible to perform serial studies over a prolonged period of time. This is a rapidly growing field and it is only possible to guess at the present time, the many applications that such studies may find in patients with various immunologic disorders.

5. Concluding remarks

Basic immunology is experiencing a period of rapid growth. New concepts originating in basic research are becoming available for application to clinical problems. The idea that the production of desired immunity and the elimination of undesired immune reactions are the basic issues in clinical immunology is acquiring new meaning, as it is becoming better understood which aspects of immunity should be reinforced and which components should be controlled or eliminated. From a combination of empirical double-blind drug trials and elaborate immunological research are emerging more effective methods of caring for patients with immunologic diseases.

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