Immunosuppressive Therapy

Proceedings of the
International Wiesbaden Symposium 1972

IMMUNOSUPPRESSIVE THERAPY
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PREFACE

With the increasing pressure to use immunosuppressive agents in clinical medicine, it seemed timely to call upon a number of experts to discuss the subject very thoroughly.

It appeared important to first sum up the experimental data which enable one to designate a compound as an immunosuppressant. When such compounds are used in human patients the situation becomes immediately more complex. Questions such as whether one is really applying immunosuppression were posed repeatedly during this symposium. The next question deals with clinical efficacy. What do we achieve in objective terms in patients with such diversified conditions as SLE, chronic active hepatitis and multiple sclerosis?

It was the aim of this symposium not just to cover a few indications, but rather to cover the main spectrum of diseases in which immune phenomena are thought to play a role in their pathogenesis and in which immunosuppressive therapy has been attempted on more than a few patients. The following clinical conditions have been covered by this symposium: SLE, kidney diseases, polyarteritis nodosa, dermatomyositis, scleroderma, cutaneous vasculitis, rheumatoid arthritis, chronic inflammatory liver diseases, blood diseases, gastro-intestinal diseases, eye diseases, multiple sclerosis, and renal transplantation. The results have been discussed with much criticism. Indeed, the purpose of this book is not to advocate this type of therapy but to state as objectively as possible what we *can* do and what we *hope* to eventually achieve with this therapeutic approach.

Immunosuppressive therapy is under special and justified attack since it constitutes an interference with normal biology, exposing the patients to a number of dangers. For this reason, one part of the meeting was devoted entirely to side effects of immunosuppressive therapy.

It is hoped that with this critical review, many clinicians will have access to information which will enable them to assess more accurately this still controversial subject.

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I. Introduction

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In recent years immunosuppressive therapy has occupied an increasing sector in the treatment of various conditions thought to be due to or aggravated by immunopathologic phenomena [15, 18, 19, 32, 41, 52, 62, 65, 76, 79, 84, 91, 94, 112, 115, 117, 141–143, 145, 148, 160, 169a, 173, 179]. Yet we are still in an experimental phase which is full of controversy. As a matter of fact, immunosuppression appears to be an unphysiological approach, since breakdown of the immune response may lead to most severe complications. Total immunosuppression is not compatible with survival. Furthermore, drugs are used which not only depress the immune response but which entail a number of hazards such as teratogenicity, cancerogenicity, and cytotoxicity.

If immunosuppression is nevertheless applied increasingly, it is for the following reasons: 1. Transplantation is proving unfeasible in almost all conditions, with the exception of transplanting organs among identical twins, because of an immunologically induced rejection of the transplant. Immunosuppression appears to be the logical approach to this problem and has indeed proved quite successful (see chapter on Transplantation by Dr Thiel, p. 117). 2. In a number of clinical conditions it was found that mild immunosuppression alleviates immunopathologic conditions without producing the related hazard to infection, i. e. without undue impairment of the host's response to microbes. 3. In various experimental autoimmune diseases as well as in the SLE-like condition of the New Zealand mice, immunosuppressive therapy proved useful in either preventing incipient disorders or in alleviating on-going disease.

In recent years, investigation of cellular and molecular events of immunization have made it possible to study different ways and means of interfering with the immune response [9, 39, 45, 154]. Today we can already see the fruits of this research in the differential application of the various immunosuppressive agents according to the type of immune response. It does seem appropriate in a symposium on immunosuppressive therapy to summarize our present understanding of the immune response in conjunction with the possibilities of pharmacological interference.

Fig. 1 summarizes today's concept of immunization. In the first phase the antigen is acting on immunocompetent cells either directly on B or T lymphocytes, or with macrophages as a mediator. This first phase can be summarized by the term "antigen cell-to-cell interaction" [31, 48, 104]. A number of agents are capable of interfering with the immune response at this level; anti-lymphocyte serum (ALS), prednisone, substances acting on the microfilamentous structure such as vincristin and cytochalasin B.

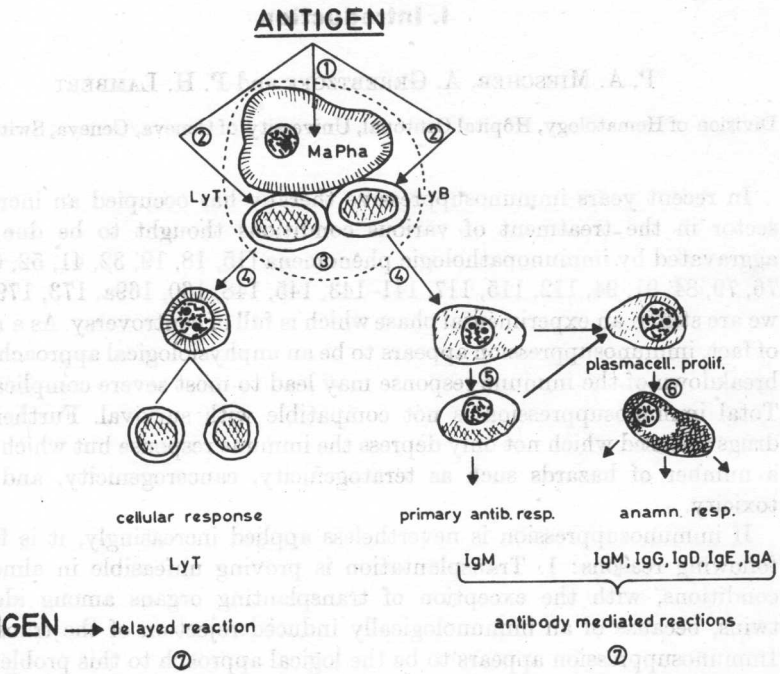

Fig. 1. Immunization mechanism indicating sites of action of immunosuppressive drugs. – *Phase I:* cell-to-cell interaction between macrophages, T and B lymphocytes. 1. Phagocytosis and processing of antigens: plant alkaloids, corticosteroids, alkylating agents, L-asparaginase, actinomycin. 2. Interaction between antigen and lymphocytes: ALS (anti-lymphocyte serum), antibodies, L-asparaginase. 3. Lympho-cytotoxic action: ALS, vincristin, alklyating agents, large amounts of corticosteroids, L-asparaginase. – *Phase II:* sensitization of lymphocytes. 4. Transformation of lymphocytes into lymphoblasts: alkylating agents, antimetabolites, antibiotics, plant alkaloids. 5. Primary IgM immune response: alkylating agents, antimetabolites. – *Phase III:* anamnestic response. 6. Antimetabolites, alkylating agents, antibiotics, antibodies, high doses of corticosteroids. – *Phase IV:* inflammation secondary to an immune reaction. 7. Anti-inflammatory agents, corticosteroids, 6-mercaptopurine.

The second phase is characterized by proliferation of lymphocytes leading to the primary immune response. With regard to the lymphocyte T immune response, this phase is sensitive to most agents which inhibit DNA, RNA, and protein synthesis. With regard to B lymphocytes, the primary IgM immune response is much more resistant to most agents. Alkylating drugs appear for the time being the most effective immunosuppressive agent in this regard.

The third phase deals with the massive proliferation of plasma cells. While the proliferation of B and T lymphocytes leads to other B and T lymphocytes which maintain full proliferative potential, the plasma cellular proliferation of the anamnestic response leads, after about 6 cell cycles, to

end-cells which are going to die upon releasing all the antibody. This proliferation is sensitive to most agents which inhibit DNA, RNA or protein synthesis.

At the present time only a limited number of immunosuppressive agents have been introduced into clinical medicine. We will summarize the most important compounds currently in clinical use as well as a few compounds which appear interesting although not used for patients:

A. Alkylating agents

Alkylating agents interact with nucleophilic centers of molecules, especially with the amino, carboxylic, thio, and phosphate groups, as well as with tertiary nitrogen. Their main site of action is located on the N^7 of guanine residue of DNA [14, 37, 57, 86, 137, 178]. They alkylate nuclear and cytoplasmic RNA, enzymes, and structural proteins. As they have at least two alkylating groups, active alkylating agents cross-link native DNA together, DNA with proteins, DNA with RNA, RNA with RNA, or RNA with proteins [63, 163, 178]. In binding N^7 of guanine in the DNA molecule, alkylating agents labilize the glucosidic bridge between base and deoxyriboside, causing depurination and chain scission. In some instances, the DNA molecule remains intact; during RNA replication it may thus produce an erroneous codon. At the cellular level, alkylating agents therefore interfere with nucleic acid synthesis and cell functions. They block the cell cycle in the G_2 phase [163, 178]. Furthermore, they may cause a miscodification of messenger, transfer, and ribosomal RNA, which in turn may lead to impairment of protein synthesis.

As immunosuppressants, alkylating agents act on the rapidly dividing cells, i. e., the small sensitized "short lived" lymphocytes [63]; they do not affect small "long lived" lymphocytes [103]. The most widely used alkylating agent both in experimental and human therapy is cyclophosphamide (Endoxan, Cytoxan). The other alkylating agents used in immunosuppression are the nitrogen and uracil mustards (chlorambucil and melphalan, an L-phenylaline mustard), dimethylsulphonates (busulfan), and polyethyleneimines (thio-TEPA, triethylenemelamine). The action of procarbazine (a methylhydrazine derivative) and mitomycin C on the immune response can also be explained by an alkylating effect [37, 155].

Alkylating agents mainly act through their lymphocytolytic effect. *Cyclophosphamide* suppresses the *primary immune response* if administered before, during, or after antigenic stimulation. It affects this primary immune response at different stages [22, 108, 136]. Maximal effect is observed in rats when injected before, or 4 days after, antigenic stimulation [163]. On the contrary, antibody production can be stimulated when this drug is administered between 11 and 7 days prior to the antigen [163]. In mice stimulated with heterologous red blood cells, the maximum effect is obtained when the immunosuppressive agent is given 1–3 days after the administration of the

antigen [39]. Cyclophosphamide limits the formation of pyroninophilic blast cells in the primary response [117]. In man, cyclophosphamide has also been found to impair immunoglobulin production [2].

Cyclophosphamide may also inhibit the *secondary immune response* and impair on-going antibody synthesis, although to a lower degree than the primary response [136, 170]. Cyclophosphamide has been shown to induce immune tolerance if administered simultaneously with high doses of antigen [1, 63, 89].

Delayed hypersensitivity is inhibited by cyclophosphamide when the compound is given during sensitization [16, 137, 143]. Similarly, contact sensitivity to inorganic metal compounds is attenuated in guinea pigs [126]. Graft survival is prolonged by cyclophosphamide in mice, rabbits, rats, and men, but not in dogs and guinea pigs [57, 137, 143].

Alkylating agents have proved effective in various experimental autoimmune diseases [50, 59, 80].

In clinical medicine cyclophosphamide is the most used compound belonging to this family. The next most used compound is chlorambucil. Alkylating drugs are essentially used in an attempt to affect the IgM immune response [140]. Indeed these agents are the most potent ones in this regard. They are little used otherwise in view of their potentially high cancerogenicity.

B. Antimetabolites

Antimetabolites are synthetic substances that have a) a structural analogy with nucleic acid bases capable of interfering with de novo synthesis of these bases or of utilization of preformed bases, and b) inhibit essential enzymes for the de novo synthesis of nucleic acid bases. Purine and pyrimidine analogs, folic acid antagonists, and analogs of glutamine are the most frequently used antimetabolites.

Purine analogs

The first synthesized purine analog was thioguanine, but synthesis proved difficult the second time, so attention was turned to the synthesis of 6-mercaptopurine (6-MP), a structural analog of hypoxanthine, which in its nucleotide form, inosinic acid, is the center point of nucleotide metabolism [43]. The imidazole derivative of 6-MP, azathioprine, is one of the most widely used immunosuppressive drugs in basic research and therapy. The sites of action of 6-MP are multiple. In entering a cell, 6-MP is converted by inosinic acid phosphorylase into its nucleotide form, thioinosinic acid. The latter is transformed into thioxanthylic acid by inosinic acid dehydrogenase (thioxanthylic acid is a ribonucleotide) [117]. Thioinosinic acid is the active substance inhibiting a) de novo synthesis by feedback inhibition and b) purine salvage pathway.

Some of the 6-MP is methylated to 6-methylthiopurine and some is converted into thioguanylic acid which is incorporated into DNA and RNA [44].