Differences among immunosuppressive agents

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SUMMARY Immunosuppressive agents have diverse (although often multiple) sites of action in the cell sequences that are involved in immune responses. New routes to selectivity are apparent at both the cellular and the biochemical level. Meanwhile, clinical work is finding new uses and more selective employment of the currently available agents.

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Immunosuppression had a spectacular debut with the finding, by Schwartz *et al*,^{31 193 194} that immunological tolerance could be produced by the administration of purine-6-thiol (6-MP) with, or soon after, a dose of bovine gammaglobulin to rabbits. The production of tolerance was closely dependent on timing of drug and antigen administration, as shown in fig 1. It also

was dependent on both the dose of antigen and the dose of drug, so that it had characteristics of the immune paralysis induced by massive doses of antigen.⁹¹ Moreover, both Schwartz *et al* and Frisch and Davies⁷³ showed that it could be highly specific. Frisch and Davies, for example, gave human erythrocytes to mice, waited two days, then gave a single dose of thioguanine and sheep red blood cells. Antibodies were produced to the sheep erythrocytes, but not to the human.

The observations of Schwartz and Dameshek¹⁹⁴ were quickly developed in two directions. They began investigations of autoimmune diseases that are still continuing and are the major focus of today's workshop. The finding also provided entry into the field of organ transplantation, then awaiting only a means of controlling the rejection of allografted organs. This field has made rapid progress; today renal allografting is regarded not as an experimental, but as a therapeutic procedure, with a high probability of long-term survival.⁵⁸ Almost from its inception, renal transplantation has involved the use of azathioprine, which had been synthesised originally as a masked form of 6-MP for anti-cancer studies,⁵⁹ and it has superseded 6-MP, not only in this, but also in the autoimmune field.^{60 92} An overview of the scope of this field is provided in table 1 which simply lists the types and numbers of clinical investigations known to be in progress in the USA.

Many of the problems and difficulties of interpretation that could be perceived in 1967⁹¹ are still with

FIG 1 The effect of timing of drug administration with respect to antigenic stimulus. Data drawn from Schwartz *et al*.^{31 193 194} In each case bovine gammaglobulin 2 mg/rabbit was administered on day 0. In panel A the drug (horizontal bar) was given before the antigen, in panel B during antibody production, and in panel C starting with antigen administration and continuing 7 days.

TABLE 1 The types and numbers of clinical investigations of autoimmune disorders known to be in progress in the USA, using azathioprine

Category	Number of trials	Total number of patients
Rheumatoid arthritis	12	260
Hepatitis	2	265
Reiter's	1	10
Graft vs host	1 (+1)	50
Multiple sclerosis	2	200
Crohn's	8	400
Myocarditis	1	10
Myasthenia gravis	?	?
Lupus (erythematosus) nephritis	?	?

us. However, striking progress has been made on several fronts and new ground is being broken on others. Before attempting to discuss these fields, it is probably pertinent to mention the numerous additional substances that have immunosuppressive activities. Most of these are still being drawn from the armamentarium of anti-tumour agents⁹¹ (table 2). It was this multiplicity of agents that prompted the question "are all immunosuppressants the same?" The short answer, of course, is "no". An attempt will be made in this paper to analyse the differences from several viewpoints and to point the way towards firmer knowledge in several essential, but possibly ancillary, areas.

The last two decades have witnessed remarkable advances in the recognition of lymphocyte types and functions. What in the early 1960's was simply a distinction between cell-borne and humoral immunity soon became a distinction between T and B lymphocytes. The importance of the thymus was elegantly shown by the experiment depicted in fig 2. Meanwhile, the past decade has seen a rapid proliferation in the recognition of various subclasses of T lymphocytes^{86 110} and factors involved in their differentiation and activities. Much of this material will be dealt with elsewhere in this workshop; however, an attempt will be made here to present some of the effects of drugs on specific cell types. A selection among such reports is presented in table 3. This is, in fact, a selection not guaranteed for immutability, since surveys of the literature reveal a dependence of such identifications on the specific conditions of the reporting laboratory. However, one must regard such identifications as a step in the understanding of the mechanisms of immunosuppression that may lead to the selective use of particular agents.

What may eventually be regarded as the most important breakthrough of all is the recent discovery that deficiencies in certain enzymes, concerned with purine intermediary metabolism, are associated with immunological defects. The first of these to be reported was congenital deficiency of adenosine deaminase (ADA) in a patient with severe combined immunodeficiency affecting both B and T cells.⁷⁸ Three years later, the same group found another

FIG 2 Documentation of the role of the thymus in the differentiation of lymphocytes. The responses of splenic lymphocytes to culturing in the presence of concanavalin A are shown. (Courtesy of Life Sciences Research Reports 1981; 11: (1). SRI international Menlo Park, CA 94025, USA.)

TABLE 3 Reports of cellular responses to immunosuppressive agents

Agent	Effects	Reference
Cyclosporin	TCGF response ↓	120
Dexamethasone	TCGF production ↓	120
Azathioprine	Inhibits RFC (T cells)	53 228
Cyclophosphamide	↓B cells, ↓suppressor T cells	128 197
Melphalan	↓Activated B cells	76
BCNU	↓Activated and resting B cells	76

↓ = decrease.
TCGF = T cell growth factor.
RFC = rosette forming cell.
BCNU = carmustin

TABLE 2 Immunosuppressive agents

A Steroids
B Alkylating agents
C Antimetabolites
(1) Purinethiols
(2) Inhibitors of dihydrofolate reductase
(3) Nucleoside analogues
D Intercalators
E Cell cycle blockers
F Enzymes
G Antibody

deficiency, of purine nucleoside phosphorylase (PNP),⁷⁷ that may eventually be even more helpful in understanding the biochemical events of the immune response. Most of the explanations of the effects of ADA deficiency revolve around the toxicity of deoxyadenosine. However, PNP deficiency is selectively toxic to T cells.⁷⁷ Deficiencies in phosphoribosyltransferases have only minor effects, although some impairment of B cell activities have

been noted.³ The explanation of the selective effects of PNP deficiency is attributable to the existence of a deoxycytidine kinase that phosphorylates d-guanosine (d-Guo), whereas in the ribo-series there is no similar enzyme. The deoxyguanylate eventually reaches the triphosphate stage and exerts a feedback inhibition of ribonucleotide reductase. The phosphorylation is the preferred route of metabolism of d-Guo in T cells, but is almost non-existent in B cells. Thus, d-Guo is highly toxic to T lymphoblastoid cell (T-LCL) lines but only mildly so to B-LCL, and not at all to B-PNP deficient.¹⁶³ These findings are summed up in table 4.

It is apparent that observations of this sort may not only lead to more precise definitions of immune response, but lay the basis for rational approaches to selective drugs.

TABLE 4 *Enzyme deficiencies affecting immune responses*

Deficiency	Lymphocytes inhibited	Biochemical abnormalities
ADA	B T	d-Ado excretion ↑dATP, ATP, cAMP
PNP	T	↑d-Guo ↑d-GTP ↓nucleotide reductase
APRT	none	2, 8-diOH-6-NH ₂ purine→
HGPRT	none	↑uric acid

Above the line, abnormalities producing major effects on immune responses. Below the line, deficiencies having at most minor effects. ADA=adenosine deaminase, PNP=purine nucleoside phosphorylase, APRT=adenine phosphoribosyl transferase, HGPRT=hypoxanthine guanine phosphoribosyl transferase, d-Ado=deoxyadenosine, ATP=adenosine triphosphate, dATP=the corresponding deoxyribose derivative, cAMP=cyclic adenylate, d-Guo=deoxyguanosine, d-GTP=deoxyguanosine triphosphate. Arrows signify increases (up) and decreases (down).