Therapeutic aspects

Immunosuppression in established humoral responses

R. F. GAGNON AND I. C. M. MACLENNAN

From the Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford

Autoantibodies and circulating immune complexes are common associated findings in inflammatory disease of unknown aetiology. In most circumstances, however, it is far from easy to demonstrate their pathogenic significance. Sometimes pathological events can be linked clearly with the presence of autoantibodies. These include antibodies found in autoimmune haemolytic anaemia and myasthenia gravis. On the other hand, in a host of other diseases the precise mechanism of tissue injury and inflammation is understood only in the vaguest conceptual terms. For this reason it is important to consider the mechanism of action of cytotoxic chemotherapeutic agents that have beneficial effects in these diseases.

Are 'immunosuppressive' agents helpful in diseases associated with autoantibodies and circulating immune complexes?

The poor prognosis of untreated neoplastic disease and the relative homogeneity of many tumour types have facilitated evaluation of the use of cytotoxic agents in these conditions. In comparison, studies of the effect of these agents in idiopathic inflammatory disease have been difficult to perform. The difficulties include (1) the ethics of using such agents in diseases that may respond to less toxic agents; (2) the difficulty of defining disease categories, since many of the disease entities merge with each other; (3) these conditions are often of a remitting and relapsing character, consequently only large controlled studies with randomised entry can be expected to provide convincing evidence of the benefit of a particular treatment policy.

Enthusiastic reports of the benefits of agents with 'immunosuppressive' properties tend to come from non-controlled, or inadequately controlled, pilot studies. Subsequent large, randomised trials often fail to confirm the early expectations. A review of the use of cyclophosphamide or azathioprine in the nephritides of systemic lupus erythematosus highlights this trend (Wagner, 1976). More recent reports

of controlled studies have not reversed the conclusion that early enthusiasm for the use of these agents was based on inadequate information (Decker *et al.*, 1975; Donadio *et al.*, 1976, 1978). Similarly, it has been reported from pilot studies that azathioprine improved the remission rate and prevented relapse in cases of ulcerative colitis (Caprilli *et al.*, 1966; Bowen *et al.*, 1966; Mackay *et al.*, 1966; Theodor *et al.*, 1968.) A subsequent substantial double-blind, controlled trial failed to confirm these findings (Jewell and Truelove, 1974).

Some controlled trials, however, have clearly shown clinical benefit from cytotoxic agents in certain inflammatory conditions. For example, Barratt and Soothill (1970) showed that cyclophosphamide given before corticosteroid withdrawal reduced the incidence of relapse in steroid-sensitive nephrotic syndrome of childhood. But trials with clear-cut results are rare, and there is considerable need for further randomised controlled trials. Two excellent recent articles clearly discuss trial design and method and indicate the size that trials have to be to show small differences (Peto *et al.*, 1976, 1977). The rest of this paper will evaluate the action of 'immunosuppressive' agents, and indicate in particular their effect on established antibody responses.

Levels of action of cytotoxic agents on humoral responses

We have stated that the role of 'immunosuppressive' agents in diseases associated with autoimmune phenomena is generally poorly understood. A fuller summary is available in an excellent review on the subject (Berenbaum, 1975).

Suppression of primary immune responses is relatively easy to achieve if the cytotoxic agent is given at the right time. Generally speaking, for maximum effect the drugs with anti-proliferative action have to be given in the period of the development of the immune response—that is, during the first few days after exposure to antigen (Levin *et al.*, 1964; Santos, 1967). Whole body x-irradiation, on

the other hand, is effective if given before exposure to antigen but may actually potentiate immunity if given later (Taliaferro *et al.*, 1952). These considerations, however, are largely irrelevant to the question of suppression of an established immune response. This clearly is the situation when a patient presents with an illness with associated autoantibodies. The action of cytotoxic agents on antibody production in established immune states must therefore be considered.

It is tempting to assume that cytotoxic agents mainly act on antibody-producing cells or their precursors. But there is increasing evidence that these are not the only or even the principal targets for modification of immune responses by cytotoxic agents. We must consider a number of different levels of action, such as their effects (1) on antigen supply; (2) on antibody-producing cells and their precursors; (3) on cells that help to initiate antibody production and cells that regulate the number of antibody-producing cells being produced; and (4) in modifying effector systems triggered by antibody.

EFFECTS ON ANTIGEN SUPPLY

Autoantibody levels tend to follow disease activity. For example, anti-DNA antibody levels in patients with systemic lupus erythematosus generally fall during remission (Hughes, 1971; Pincus *et al.*, 1971). Such falls may be associated with regression occurring in the absence of treatment with 'immunosuppressive' agents. This effect may well be associated with changes in the antigen supply. Conceivably cytotoxic drugs directly modify the form or effect of antigen presented to the immune system. In this respect the effect of the DNA analogue 5-iodo-2' deoxyuridine on virus infections (Jaffe and Lehner, 1968; Juel-Jensen, 1969) is interesting in view of the suggested viral implication in the pathogenesis of the lupus syndrome of New Zealand black mice (Dixon *et al.*, 1974).

EFFECTS ON ANTIBODY-PRODUCING CELLS, THEIR PRECURSORS, AND REGULATORY CELLS

Studies of the appearance and demise of plasma cells in the spleens of rodents during primary immune responses to sheep erythrocytes indicate that the life span of these antibody-producing cells is from a few hours to a day or two. Consequently continued maturation of B cells to plasma cells is needed to maintain antibody production. Experimental investigation of the action of 'immunosuppressive' agents during established immune responses suggests that this process is remarkably difficult to disrupt. For example, rabbits receiving 600-700 rads *x*-irradiation four days after the induction of a secondary

antibody response to sheep red blood cells were found to produce normal secondary antibody responses (Taliaferro *et al.*, 1952). Similarly, single injections of cyclophosphamide given to rats during established secondary responses to chicken red blood cells actually augment antibody production in the spleen measured 10 days later. This effect, which exemplifies a large number of studies where cytotoxic agents potentiate antibody production, was seen with drug doses as high as 400 mg/m² (Gagnon and MacLennan, 1979).

In both these experimental situations the drop in the number of total body lymphocytes was such that a sizable proportion of B cells must have been lost. This type of observation leads to the conclusion that the number of antibody-producing cells or their precursors in normal antibody responses is not limiting and that the amount of antibody production is determined by other factors. Probably these potentiation effects are due to disruption of homeostatic or suppressor elements. The number of papers on immunological homeostasis has grown rapidly but there are few data available on the direct action of cytotoxic agents on cells regulating established antibody responses.

The two examples quoted above, showing no effect or even potentiation of established antibody production, relate to single exposures to cytotoxic agents. With long-term administration of 'immunosuppressive' agents some degree of suppression can be achieved, but this is not rapid nor very profound. For example, Hoffsten and Dixon (1974) prevented the primary response of mice to keyhole-limpet haemocyanin (KLH) with irradiation or cyclophosphamide given before the antigen. But, once established, the immune response was very resistant to suppression by drug. Nine months' administration of cyclophosphamide depressed but did not abolish the anti-KLH response. As in the Taliaferro experiment, *x*-irradiation in this system given after antigen potentiated rather than depressed established antibody production.

We have found small but significant depression of the anti-chicken red blood cell response in mice after 8 weeks of daily cyclophosphamide treatment at 10 mg/kg/day. Within 20 days after stopping the drug, however, the response had returned to that of untreated immune controls (Gagnon and MacLennan, unpublished results). Azathioprine given continuously can similarly depress but not abolish the maturation of B cells to plasma cells. This has been assessed by measuring the number of plasma cells in the lamina propria of the gut on rectal biopsy. The rate of suppression here was seen to be very slow, and gradual depletion was seen throughout 12-months' observation (Campbell *et al.*, 1976). The

number of gut plasma cells recovers rapidly after drugs are withdrawn (Campbell *et al.*, 1974).

The conclusion of this section, therefore, is that the experimental evidence suggests that 'immunosuppressive' agents have a relatively small effect on established antibody production by direct action on antibody-producing cells. Potentiation of antibody production, on the other hand, may not necessarily be undesirable in some diseases associated with immune complexes and autoantibodies. Soothill and his colleagues have argued that cytotoxic drugs may alter the quality of antibody produced in such a way as to result in clearance of circulating immune complexes (Soothill and Steward, 1971; Steward *et al.*, 1973).

ACTION OF IMMUNOSUPPRESSIVE DRUGS ON EFFECTOR MECHANISMS TRIGGERED BY ANTIBODY

This is an important level of action to be considered when one is assessing the overall action of 'immunosuppressive' agents in diseases associated with auto-antibody production. Hawkins and Cochrane (1968) emphasised this effect of 'immunosuppressive' agents in reporting their studies on the role of polymorphonuclear leucocytes in the induction of glomerular damage in experimental nephrotoxic nephritis. They showed that polymorph depletion by nitrogen mustard largely protected rabbits from the nephrotoxic effect of anti-basement-membrane antibody. Later studies have confirmed these results, and similar therapeutic effects have been obtained with the use of anti-polymorph serum (Naish *et al.*, 1975). Although the results are not compelling the effect of corticosteroids on neutrophil migration into tissues may be a significant action of this drug in preventing tissue injury (Rebuck and Mellinger, 1953).

In a different effector system Campbell *et al.* (1976) showed that azathioprine given over a period of 12 months caused a gross selective depletion of K cells in man. Similar depletion was seen in children with acute lymphoblastic leukaemia when they had been on long-term treatment with mercaptopurine and methotrexate (Waller *et al.*, 1977). In both situations K cells recovered within two to six weeks of stopping drugs.

Conclusions

(1) There is a continued need for adequate controlled trials of the action of agents with 'immunosuppressive' properties in human disease. (2) The direct effect of these agents on antibody-producing cells or their precursors is relatively small. (3) Cytotoxic agents can potentiate antibody production in established immune states by selective disruption of

homeostatic mechanisms. (4) 'Immunosuppressive' agents may be effective clinically by direct action on antigen source or effector systems triggered by antibody.

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