THE MODE OF ACTION OF METHOTREXATE UPON INSULIN ANTIBODY FORMATION IN GUINEA PIGS

By

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ABSTRACT

THE MODE OF ACTION OF METHOTREXATE UPON INSULIN-ANTIBODY FORMATION IN GUINEA PIGS

A method for quantitative radio-immunoassay of insulin antibodies was first devised, employing alcohol precipitation to separate free from antibody-bound insulin. This technique proved to be simple, specific for insulin and pro-insulin, practical, and sensitive, and yielded reproducible results. Secondly, immune responses to insulin in guinea pigs were studied. Under comparable experimental conditions, bovine insulin was shown to have approximately the same antigenic but much more immunogenic activity than porcine insulin. The response to a single injection of antigen varied from one animal to another, but if groups of six or more were used, and if a control group of animals was included in each experiment, the mean response was sufficiently reproducible to all for quantitative study and comparison of the effects of drugs which have been shown to affect immune responses in various systems.

Primary antibody formation was completely suppressed by methotrexate in non-toxic doses, but only partially affected by cyclophosphamide and prednisolone. Purine analogs (6-mercaptopurine and imuran) were ineffective

in relatively toxic doses. These drugs had no significant inhibitory effect upon antibody formation once an immune response had been established. Antibody produced by immunized animals under normal conditions and under the influence of 6-mercaptopurine, imuran and sub-inhibitory doses of methotrexate was exclusively of the 7S (IgG) class.

Methotrexate maximally inhibited antibody formation when administered daily for 30 days, between the 5th and the 10th days (Phase I), or between the 20th and the 25th days (Phase III) after initial antigen injection; or for five days after secondary antigen injection (Phase II). It is suggested that methotrexate inhibits the immune response (a) by suppressing the early cellular proliferation of antigen-stimulated cells (Phase I and II) without affecting induction of immunological memory; and (b) by reducing the actual antibody synthesis which would otherwise be occurring at a very rapid rate in the later stages of the primary response (Phase III). It is concluded that methotrexate has no suppressive effect upon an established immune response because at this stage, little or no cellular proliferation is occurring among antibody-forming cells and antibody synthesis among the cells is occurring at a very slow rate, similar to that

of normal serum proteins.

The immunosuppressive action of methotrexate was completely and qantitatively prevented by simultaneously injected folinic acid factor (FLA) in all phases (I, II and III). This suggests that these actions are mediated through the inhibition of synthesis of folic acid coenzymes from the native vitamin.

Thymidine and a purine nucleoside (deoxyadenosine or guanosine) was administered together and concurrently with methotrexate, completely reversed the inhibitory action of the antifolate in Phases I and II. Adenosine, guanosine but not thymidine alone were each able to prevent the effect of methotrexate in Phase III. Methotrexate may therefore suppress immune responses (a) by inhibiting synthesis of nucleic acids during the early stages of primary and secondary responses, this inhibition resulting from depletion of sources of thymidylate and purine nucleotides; and (b) by inhibiting actual synthesis of antibody during the later stages of the primary response, this effect being possibly due to temporary depletion of the source of purine nucleotides.