Immune supression and histophysiology of the immune response. Mode of action of 6-mercaptopurine, nitrogen mustard, cyclophoshamide and cortisone acetate
Broek, Arie Adriaan van den
SUMMARY

In the experiments described in this thesis the effects of 4 different cytostatic agents on the immune response were studied in a standardized and kinetically more or less well defined experimental system.

This system consists of the intravenous injection of Salmonella java vaccine followed 6 or 12 hrs later by the administration of some homologous hyperimmune serum. This latter procedure brings about some sort of "synchronization" of the cellular events representing the immune response. This results a.o. in a better resolution of the H-agglutinin titer curves. In additions the immune response is largely restricted to the spleen. Skin allograft responses were used to study the effects on cellular immunity.

6-MERCAPTOPURINE was given to rabbits in a course of 9 daily injections (6 mg/kg b.w./dd) on various moments before and during the anti Salmonella java H-agglutinin response, "synchronized" by a small quantity of homologous hyperimmune serum injected 6 hrs after intravenous vaccination.

When the whole course was administered prior to the antigen (-13 till -15 days or -9 till -1 day) neither depression nor enhancement of the antibody production was observed. Histologically no damage of marginal zone cells, the presumable plasmacell precursors, was found but changes in the follicular cell population suggested a temporary block of precursor cell supply.

A suppressive effect on both IgM and IgG production was observed when 6 MP was given from -3 till +5 days, from simultaneously till +8 days, from +1 till +9 days and from +2 till +10 days.
with respect to the antigen administration. A temporary interruption of the late, slow IgG log phase was found when 6 MP was given from +10 till +18 days.

Skin allograft rejection was slightly retarded by a 6 MP course given from simultaneously till +8 days and +4 till +12 days with respect to grafting.

Histologically, mitotic death of proliferating immature plasma cells, germinal centre cells and cells involved in the specific cellular (allograft) response was the only visible injury.

These results are discussed and it is concluded that a net mitotic death of appr. 50% would account for the immune suppression observed and for the histological changes with respect to the precursor cell population and its production.

NITROGEN MUSTARD, when given as a single intramuscular injection (2 mg/kg, b.w.) before the administration of Samonella java vaccine only slightly retarded and moderately suppressed the H- agglutinin response. When similarly tested with the “synchronized” Samonella java antibody response the drug was found to cause a loss of immunological responsiveness for some 24 hrs followed by a gradual return to normal in appr. 5 days. CYCLOPHOSPHAMIDE (100 or 200 mg/kg b.w.) given before the antigen did not significantly affect immunological responsiveness or antibody peak titers when tested in the “synchronized” Salmonella java system.

Histologically the two alkylating agents were found to have qualitatively similar effects on non-antigenically stimulated lymphoid tissue viz. causing interphase death of marginal zone cells and follicular small lymphocytes within some 12 hrs. Nitrogen mustard completely killed and cyclophosphamide only partially destroyed the two cell populations which presumably contain the antibody forming cell precursors. It was concluded that the immunological effect of these substances, when given before antigenic stimulation, depended on the degree of direct histological damage to the non-thymus derived, follicular (coronal) lymphocytes and marginal zone cells. This type of immune suppression is comparable to that observed following sublethal doses of total body X-irradiation given prior to antigenic stimulation.
When given after administration of the antigen nitrogen mustard and cyclophosphamide severely affected the "synchronized" type of Salmonella java H-agglutinin response and in a perfectly identical way, but only when administered during a very restricted period - from 1 through 3 days - after the antigen. With the doses used an almost complete suppression, or immediate stop, of IgM and so-called "initial" IgG synthesis was observed when the drugs were administered 1 (1½) or 2 days and 3 days respectively after the antigen. The late, slow exponential rise of IgG antibody was not itself affected, but for its starting level which represents the "initial" IgG synthesis.

Histologically no direct destruction of mature or immature plasma cells was observed; likewise mitotic death of these cells was not seen to any significant degree. However, a characteristic vacuolization of the basophilic cytoplasm and fading of nuclear chromatin was found in all immature plasma cells. Mitotic activity of these cells was minimal. It was concluded that these changes constituted the morphological correlate of the immune suppression observed, and presumably represented a disturbance of nucleic acid metabolism affecting more or less exclusively the differentiation of plasmablasts toward mature plasma cells.

In addition considerable histological damage was observed in active germinal centres, 6-12 hrs after both nitrogen mustard and cyclophosphamide. Lymphoid germinal centre cells in various stages of maturity apparently were destroyed in interphase and mitotic activity of surviving cells was reduced. Within 3-4 days, however, germinal centre activity was resumed.

A course of 7 daily intramuscular injections of CORTISONE ACETATE (20 mg/kg. b.w. per day each) was found to significantly depress H-agglutinin formation in the "synchronized" Salmonella java antibody response only when given just before antigenic stimulation. Within the limits of experimental conditions obtaining, cortisone acetate did not affect the induction process, transformation, differentiation or multiplication of the antibody forming plasma cells.

No effect on skin allograft rejection was observed when the course of cortisone acetate was given either before or after grafting.
Histologically four main effects on the lymphoid tissue were observed:

i. a moderate destruction by interphase cell death of various kinds of lymphocytes and other lymphoid cells mainly during the first days of cortisone treatment;

ii. a pronounced shift of the follicular lymphoid cell population from small coronal lymphocytes to marginal zone cells in the course of the treatment;

iii. a complete inactivation and lymphocyte depletion of germinal centres, both when induced by endogenous stimuli as e.g. in the appendix and when evoked by the administration of antigen.

iv. a total inactivation and lymphocyte depletion of the thymus cortex.

Local irradiation of a lymph node or the spleen during or after cortisone treatment demonstrated that peripheral lymphoid cell traffic was not affected.

This was obtained for thymus-derived lymphocytes homing in thymus dependent areas as well as for non-thymus derived follicular (coronal) lymphocytes from which the marginal zone cells originate. This latter - non-thymus derived - class of lymphoid cells, which earlier experiments had demonstrated to represent antibody forming cell precursors, was recently shown to be germinal centre derived.

Total body X-irradiation (450 rads) with the appendix shielded - so as to protect large numbers of active germinal centres - combined with cortisone treatment then confirmed that cortisone stopped the production of these germinal centre derived lymphoid cells rather than destroy them.

It is concluded that the functionally and morphologically most relevant effect of cortisone on lymphoid tissue is its interfering with the production of both thymus derived and non-thymus derived immunocompetent lymphoid cells. The hypothesis is brought forward that these two effects are due to a recruitment block of bone marrow derived lymphoid stem cells.