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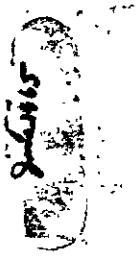

CONTROL OF SECONDARY SYNDROME FOLLOWING WHOLE BODY IRRADIATION TREATMENT WITH BONE MARROW TRANSPLANTS

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- Annual Summary Report -

a) Summary of the work accomplished during the period of the report

We have continued our study of the prevention of the secondary syndrome by the use of immunosuppressive drugs ; these drugs have been chosen for in vitro and in vivo tests as described in our previous report. The drugs were used a) to attempt to reduce the immune reactivity of allogeneic cells used to restore irradiated recipients ; b) conjointly with the administration of histocompatibility antigens from the recipient strain/ in the form of cells or of cellular extracts in order to induce a specific tolerance in the immunolofically competer cells of the donor with regard to the recipient. The schedule of drug administration was chosen so that the scheme could be later transferred for clinical trials in man ; one-sixth of the LD 50 (based on toxicity tests over six days) were given for 6 days. The results of these tests were negative. The drugs used azathioprine, mytomicin C, actinomycin D, did not reduced in any detectable way, the graft versus host reaction which followed the administration of cells from donors that had been treated in this way when given to irradiated recipients.

We have also tried to treat the graft versus host reaction by giving to the allogeneic chimera immunosuppressive drugs.

Positive results have been obtained in mice and in monkeys. We have applied this method to treating human beings ; these patients were suffering from acute leukaemia and were given a total body dose of irradiation of 800 rads, and this was followed by injection of 3-6 x 10^{10} nucleated cells that were aspirated from bone marrow of allogeneic donors, chosen for their compatibility with the patients in relation to their ABO and HLA antigens ; these chimeric patients were then either left without any immunosuppressive therapy or treated with amylmethotrexate given early in a dose of 1-3 mg/kg or by cyclophosphamide given later in doses of 10 mg/kg, or by a combination of these two drugs, or by horse-anti-human-anti-lymphocyte antisera. The results of these forms of therapy are shown in Table 1. The total number of results are too small to allow firm conclusions to be drawn, but it seems that no spectacular improvement was seen in the patients who had received these treatments, compared to those that were left untreated.

TABLE 1

ATTEMPTS TO CONTROL THE SECONDARY SYNDROME AFTER BONE MARROW GRAFTING

Drugs	Acute Secondary Syndrome	Subacute Secondary Syndrome	Absence of Secondary Syndrome
Methotrexate	2	3	2
Cyclophosphamide	1	0	0
Methotrexate + cyclophosphamide	1	1	0
Antilymphocyte sera	1	0	0
Total number of patients treated	5	4	2
No treatment	5	3	5

We questioned whether this failure was due to the ineffectiveness of the immunosuppressive drugs ; this would have been surprising, or was it due to processes which were opposed to the action of these treatments. The administration of immunosuppressive drugs to a hematopoietic chimera could have a number of possible consequences. A non-specific depression of the graft versus host or host versus graft immune reactions could be obtained, or there could be the induction of a specific tolerance by the graft to the recipient antigens with it is in contact at the time of being treated by the immunosuppressive drug ; or there could be a breakdown of tolerance which was being established by the graft vis-a-vis the recipient.

We have tried to study this last hypothesis in animals. We have examined whether it is possible to break down, by the use of cytostatic drugs, a specific immune tolerance established in the rat for sheep red cells that had been given during their immunological immaturity. The initial results in these experiments are shown in Table II ; it can be see that the hypothesis is not contradictory to these results ; antimitotic drugs, corticosteroids, and anti-lymphocytic antisera that had been used for the treatment of the secondary syndrome of allogeneic hematochimeras were able to break an immune tolerance.

TABLE II

THE BREAKDOWN OF IMMUNE TOLERANCE TO SHEEP RED CELLS IN THE RAT

BY ANTIMITOTIC DRUGS		BY A NON SPECIFIC ADJUVANT		BY OTHER FORMS OF IMMUNOSUPPRESSION	
Cyclophosphamide	4/14	B.C.G.	0/12	Irradiation	19/20
6-Mercaptopurine	5/10	Freund's adjuvant	0/8	Anti-rat anti-lymphocyte antiserum	3/5
Methotrexate	8/13			Cortisone	1/17

+ Number of animals, following immunisation with sheep red cells, have a serum heterohaemagglutinin titre greater than 1/20 / number of animals treated.

b) No particular difficulties.

c) Our clinical experience has shown us that in man the secondary syndrome of the reaction of graft versus host caused by allogeneic bone marrow given to irradiated subjects can be systematically considered to be of two main types. There is an acute syndrome of early onset

in which it seems that the proliferation of the immuno-competent graf cells play an essential role in the pathophysiology of the syndrome. On the other hand, there is a chronic syndrome of slow onset where th immunologically competent systems of the host and of the graft are depressed by mechanims, which we have described in mice. The essentia feature of the patho-physiology of this chronic syndrome is a non-spe fic immune insufficiency. The acute syndrome seems to be able to be avoided by a better selection of the donor in relation to the recipie taking into account the ABO and HLA antigens, these have to be determined with considerable care at the time of grafting. On the other ha the chronic secondary syndrome seems to be almost inevitable. It does seem to be able to be treated by immunosuppressive drugs and we are turning our attention towards attempts to find ways to restore the im competence of the allogeneic hematopoietic chimera during this period of chronic secondary syndrome - that is to say, in a state of non-spec fic immune insufficiency.

We have now commenced an experiment trying to study the different possibilities of restoring immune capacity. The basic desig of the experiment is as follows. (F1(CBA x C57Br) ($H\text{-}2^k$ x $H\text{-}2^k$) mice were irradiated with 1000 rads and, on the same day, given 2×10^7 bon marrow cells from F1 (DBA/2 x C57B1/6) ($H\text{-}2^d$ x $H\text{-}2^b$) mice. In this particular combination, the mortality that is related to chronic secon dary syndrome does not commence until the twentieth day after irradiation.

On the fifteenth day after irradiation the animals are eith left without treatment, or treated with one of the followinf metho 1) a further injection of bone marrow cells from the donor strain, the animals were given $3. \times 10^7$ bone marrow cells (DBA/2 x C57Bℓ / 6) intravenously ; 2) a heterotype graft of a thymus isogeneic with the donor : the animals were given during the same time, by an intraperitoneal inoculation, using a trocar, 3 - 5 thymuses cut into small fragments from (DBA/2 x C57Bℓ/6) donors aged between 4-10 days old ; 3) a thymus graft allogeneic to both donor and recipient : these animals were treated similarly to the preceding group but the donors were Balb/c ($H\text{-}2^d$) mice of the same age ; 4) injection of an RNA extract from immunologically competent cells of the donor.

In this experiment spleen cells from adult (DBA/2 x C57B1/ donor mice were used. The RNA was extracted from the cells using tl Scherrer and Darnell method (B.B.R.C., 1962, 7, 486), the fraction extracted at 65°C was used in these experiments. Each chimera was injected with 1mg of the extract. This was either given by intraperitoneal injection or into the thoracic duct. The effectiveness of the restoration of immunity afforded by this RNA was observed in the following manner. The treated chimeras were given one of the followin injections of cells intraperitoneally, either 10^5 splenic cells from (DBA/2 x C57B1/6), or 10^5 spenic cells from (DBA/2 x C57B1/6) which had been incubated for one hour at 37°C in tissue culture medium 199 with 1mg of RNA extracted from spleens of isogeneic mice of (DBA/2 x C57B1/6), from whom the spleen had been removed four days after their immunisation by 5 10^8 sheep red cells.

The animals had been treated this way and those that had been left without treatment were then submitted to a further group of tests : 1) a test of their length of survival : 2) a detailed study of the main forms of immune reaction against sheep red cells, observations of the immune reaction against skin grafts of donor type (DBA/2 x C57B1/6) and allogeneic in relation to the recipients and to the donors Balb/c ($H\text{-}2^d$) ; study of delayed hypersensitivity reactions obtained by the injection of three types of antigens into the footpads candida, mumps antigens and BCG. This scheme of experiments has alread commenced but the work is insufficiently advanced to enable us to repo the results at this stage. We will be describing these results in our next report.

d) During this period, Professor MATHE and AMIEL participated in the Conference on "La Greffe de Moelle Osseuse et la Physiologie des tissu hematopoëtiques" organised in Paris, and in the Conference on " Histocompatibility testing 1967" organised in Torino.