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ANNUAL SUMMARY REPORT

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

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ABSTRACT

1) The effect of storage in the presence of antimetabolic agents and steroids on haematopoietic cells.

Methods allowing the measurement for each compound the percentage inhibition on cell growth and cell destruction in relation to the dose given in vitro have been worked out. The Brent and Medawar test in vivo, and the study of the production of haemolysins by isolated cells in a solidified medium have been adapted for use in the current research programme.

2) Investigation of the prevention or cure of the secondary syndrome which complicates grafting of allogeneic hematopoietic cells.

a) The clinical characteristics, haematological picture, and the biochemical and histological aspects of the secondary syndrome in man that follows a graft of allogeneic haematopoietic cells has been investigated in 9 patients. The possibility of an early onset of the secondary syndrome has been demonstrated. The possible lines of therapy have been studied. A preventive treatment of this syndrome in man appears to be a possibility, it is of particular importance to utilize multiple donors and give early treatment with amethopterin.

b) A syndrome identical to the secondary syndrome follows the injection of leucocytes obtained from donors with chronic myeloid leukaemia when they are injected into recipients with bone marrow aplasia caused by leukaemia of haemosarcomata.

c) It has been shown that the cells which participate in the reaction of the graft against the host at the onset of the secondary syndrome have an intrinsically high radioresistance. Prior contact with the histocompatibility antigens does not increase this radioresistance.

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Comparaison de la radiosensibilité, avant et après leur contact avec l'antigène, des cellules immunologiquement compétentes intervenant dans la réaction du greffon contre l'hôte.
MATHE G. et AMIEL J.L. C.R. Acad. Sc., à paraître. 1965.

Les divers aspects du syndrome secondaire compliquant les transfusions de moelle osseuse ou de leucocytes allogéniques, chez des patients atteints d'hémopathies malignes.
MATHE G., SCHWARZENBERG L., DE VRIES M.J., AMIEL J.L., CATTAN A., SCHNEIDER M., BINET J.L., TUBIANA M., LALANNE C., SCHWARZMANN V., NORDMANN R. J. Europ. Cancerol., 1965 à paraître.

Les transfusions de globules blancs.
SCHWARZENBERG L., MATHE G., GROUCHY J.de, NAVA C.de, VRIES M.J.de, AMIEL J.L., CATTAN A., SCHNEIDER M., SCHLUMBERGER J.R.
Israel J. Med. Sc., 1965. à paraître.

BODY OF REPORT

Under the terms of our contract research has been made on the following two topics (1) the effects of different types of storage in the presence of antimetabolic drugs and steroids on haematopoietic cells. (2) Research into the prevention and treatment of the secondary syndrome which complicates the grafting of allogeneic bone marrow.

I. THE EFFECT OF STORAGE IN PRESENCE OF ANTIMETABOLIC AGENTS AND STEROIDS ON HAEMATOPHOIETIC CELLS.

Initial experiments were made to determine the dose effect relation for each drug by studying the inhibition of growth and cell destruction of KB cells cultivated in Eagle's medium. These doses were then employed for the experiments in which the effects of these drugs on myeloid and lymphoid cells stored for various lengths of time, at different temperatures in protein containing and protein free media were compared. The effects induced by storing the cells under these different conditions were then investigated by several tests of cellular function in vivo. The first test is the determination of the numbers of cells that can restore the myeloid tissues of 50 % of isogenic mice given a lethal dose of irradiation. The second test is the estimation of the dose of semi-allogeneic lymph node cells that will kill 50 % of F1 recipient, which had been irradiated at a sub-lethal dose. The third test is an application of the Brent and Medawar test, here the effects of intra-dermal injection of the treated mice lymphocytes into heterospecific recipients (hamsters). We also test for the production of haemolysins against heterospecific red blood cells. In this test we employ the technique of Jerne, Ingraham and Bussard using red cells in a solidified medium with added complement. We have been engaged on these experiments for the past

few months and we propose to report the first results in our next report.

II. INVESTIGATION OF THE PREVENTION OR CURE OF THE SECONDARY SYNDROME WHICH COMPLICATES GRAFTING OF ALLOGENEIC HAEMATOPOIETIC CELLS.

We have studied the secondary syndrome in man, starting with our experience of grafting allogeneic haematopoietic cells in patients with acute leukaemia who have been irradiated. A similar syndrome was observed in patients suffering from malignant blood disorders who had undergone a spontaneous or drug induced bone marrow aplasia and were transfused with leucocytes obtained from chronic myeloid leukaemic patients.

A. The secondary syndrome after allogeneic bone marrow grafting in man.

We have observed this syndrome in 9 of 14 patients suffering from acute lymphoblastic leukaemia who had been given total body irradiation at doses between 750-850 rads and then received allogeneic haematopoietic cells. The details of the irradiation and marrow transfusions have already been described. Five of these cases are as yet unpublished.

This experience has allowed us to discern the characteristics of the secondary syndrome in man. The key clinical points are, fever, which remains after the restoration of the level of the circulating granulocytes, a constant erythrodermia, digestive disturbances with anorexia, vomiting, diarrhoea and an overall intestinal insufficiency. We have noted that there is a marked susceptibility of these patients to infections particularly viral and fungal.

Haematologically, the main feature is the contrast between the restoration of the granulocytes either complete or only of the medulla and an aplasia of the lymphoid tissue with lymphocytopenia.

We have also established that there may be a marked eosinophilia and noted the presence of hyperbasophilic cells in the marrow.

The main histological signs are an aplasia of the lymphoid tissue, characteristic skin lesions with hyperkeratosis of the epidermis, disappearance of all the intestinal crypts to the level of the ileum and frequent hepatic necrotic lesions with infiltration by hyperbasophilic cells.

The biochemical disturbances, a part from the intestinal insufficiency are very variable, most frequently hypogamma globulinaemia with levels in the range of 500mg / 100 ml are observed, frequently there is an increase in the serum enzymes of hepatic origin.

We have found that the secondary syndrome is liable to be very early in onset in man, which is similar to this syndrome in primates. Three of our patients died before the restoration of the myeloid tissue was complete.

The clinical pattern of the secondary syndrome, its histology, haematological and biochemical lesions in man is very close to that which is noted in animals, especially the primates. The early onset of the secondary syndrome, which we have described is most important to be aware of, for it can start before there is evidence of the restoration of the granulocytes in the peripheral blood. The histological lesions that we have described enable to contrast the deaths from acute secondary syndrome to those which are due to a simple failure of the bone marrow graft.

We have based our symptomatic treatment on the basis of these physical signs that we have observed in this syndrome. We have shown

to counteract the haemorrhagic syndrome the only effective methods would appear to be the giving of epsilon-amino-caproic acid at doses up to 1 g/kg body weight daily, and above all the use of daily platelet transfusions. Delta-1-cortisone can also be given with these treatments.

The main risk to the patient during the secondary syndrome is infection, of bacterial, viral or fungal origin, which can occur despite the restoration of the granulocytes. Treatment should attempt to keep the patient in an aseptic condition, this can be achieved in a special ward, such as the one we have available to us in the "Institut de Cancérologie et d'Immunogénétique". The patient should remain in the aseptic ward until the signs of the secondary syndrome have disappeared, that is until there is restoration of the circulating lymphocytes, this may involve a stay of several months. The administration of high doses of gamma globulin and mycostatin is of systemic benefit. Antibiotics should not be given until a pathogenic organism has been isolated and then it should be given according to the sensitivities. We have shown that repeated exchange transfusions are not able to increase the granulocyte count or to ameliorate a pre-existing infection.

Arising from these observations we have been lead to conduct experiments in animals to attempt to find a method of preventing the severe secondary syndrome. The first trial was the use of haematopoietic graft that had been incubated at 37°C for 2 hours in a medium containing no protein, this incubation reduced the incidence of the secondary syndrome without altering the restorative effect of the haematopoietic graft in mice. A trial of this type was tried in man, but it was a failure as the injection of the haematopoietic cells was not followed by a take of the graft. Restoration of this patients bone marrow was eventually accomplished by an isogenic graft from her monozygotic twin. The second of these studies consisted in the utilization of many donors, there being a spontaneous selection of the

closest related donor. The first trials of this type of therapy were followed by a secondary syndrome, although of both early in onset and severe was able to be controlled. The third line of research was the administration of a course of amethopterine of 0,5 mg/kg body weight on the day of injection and of 0,2 mg/kg body weight on the 3rd, 5th, 7th and 9th days after the injection of hæmatopoietic cells. In the patient ^{to whom} this regime of treatment was given we observed a myeloid restoration without any clinical or hæmatological indications of a secondary syndrome.

B. The secondary syndrome after the transfusion of leucocytes.

33 patients suffering from acute leukaemia or hæmato-sarcomata with myeloid aplasia of either spontaneous or drug induced types have been injected with leucocytes obtained from donors with chronic myeloid leukaemia. The quantities of leucocytes injected varied between $2 \cdot 10^{10}$ to $9 \cdot 10^{11}$, and the number of transfusions between 1 and 9.

In 7 of these patients we have observed following the transfusion the onset of a syndrome which recalls all the features that we have previously described to occur following the injection of allogeneic bone marrow. The clinical signs, fever erythrodermia, digestive tract disturbances were the same. In two of these patients who died, post-mortem studies showed histological lesions closely resembling those seen in the secondary syndrome. The complications arose when cells that had been stored in dimethyl-sulphoxide or glycerol at -70°C or at -196°C were transfused, as well as when freshly taken leucocytes were transfused. The two patients exhibiting the most severe reactions were both suffering from Hodgkin's disease. It was observed that the secondary syndrome that follows the transfusion of leucocytes appears to exert an adoptive immunotherapeutic effect on the hosts leukaemia, similar to that following transfusions of allogeneic bone marrow, although it was of a lesser degree. 8 of the patients underwent a remission following this treatment of whom 5 had complete re-

missions and this was without any chemotherapy or hormone therapy.

C. Comparison of the radiosensitivity by immunologically competent cells in graft versus host reaction before and after contact with antigens.

Several studies have shown that the secondary responses to antigenic stimulation are not less radiosensitive than primary responses. However, this sensitivity has been studied less frequently during the course of immune reactions that lead to production of serum antibodies. We have studied the problem of the radiosensitivity of murine immunologically competent cells using as a test system the reaction of a graft of lymph node cells against a semi-allogenic irradiated recipient.

164 F1 mice (C57Bl/6 × DBA/2) aged 3 months were irradiated at 500 rads and given 10^7 bone marrow cells from adult C57Bl/6 donors and the same sex, they were divided into 5 sub groups according to the following scheme. Group A, the lymphoid cells were from C57Bl/6 : not irradiated (groupe A I, 13 mice) ; irradiated at 200 rads for two hours before taking from the donor (A II, 18 mice), at 400 rads (A III, 17 mice), at 600 rads (A IV, 20 mice), at 800 rads (A V, 20 mice).; group B, the donors were F1 (C57Bl/6 × DBA/2) who had been given five days before taking the cells an irradiation of 950 rads and 10^7 bone marrow cells and 2.5×10^7 lymph node cells from C57Bl/6 mice. These donor animals were either not irradiated (B I, 15 mice) or irradiated two hours before taking the cells with 200 rads (B II, 8 mice), at 400 rads (B III, 16 mice), at 600 rads (B IV, 17 mice) at 800 rads (B V, 20 mice).

The radiation was made under the following conditions : 200 Kv, 12 mA, 50cm distance, 0,5 Al, 0,5 Cu. The survival of the animals was studied up to 100 days.

The results of these experiments are summarized in Table 1.

In both the A and B groups of animals there was a reduction of the secondary syndrome and a prolongation of duration of survival which was related to the dose of irradiation received by the transfused lymph node cells. The comparison by probit analysis did not demonstrate a statistically significant difference between the radiosensitivity of the lymph node cells used in these two groups.

The reaction of the graft versus host was confirmed to be an immunological reaction that is particularly radioresistant as we have previously observed. However, a prior contact in vivo with the allogeneic antigens did not seem to increase the radioresistance of the cells taking part in this reaction. The radioresistance would seem to be an intrinsic property of these cells and not acquired as a result of contact with the antigen as occurs with lymphocytes in vitro.

These results appear to give a good reason to conclude that the immunization of the graft versus host and in general the immunization of grafts involves cellular mechanisms other than those of the transformation of lymphocytes or of the development of an increased radioresistance.

TABLE 1

	GROUPE A			GROUPE B		
	Souris mortes avant le 100e jour	Souris survivant au-delà du 100e jour	Médiane de sur- vie des souris mortes	Souris mortes avant le 100e jour	Souris survivant au-delà du 100e jour	Médiane de sur- vie des souris mortes
Sous-groupe I : 0 rads	13	0	9 j	11	4	14 j
Sous-groupe II : 200 rads	18	0	17 j	8	0	21 j
Sousgroupe III : 400 rads	15	2	15 j	8	8	28 j
Sous-groupe IV : 600 rads	16	4	22 j	6	11	31 j
Sous-groupe V : 800 rads	13	7	19 j	8	12	34 j