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European Atomic Energy Community - Euratom

Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek - TNO

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Annual Report 1966

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Report prepared at the TNO
Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek,
Rijswijk - Netherlands

Radiobiology Institute

Association No. 062-66-1 BIAN

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SUMMARY

For the contract period 1966-1967 it was proposed to concentrate on methods aimed at the selection of bone marrow donors with maximum histocompatibility with regard to the irradiated recipients.

Most of the work carried out during 1966 has been on the typing of leucocyte antigens, as is currently being pursued in man.

A major advance in the control of secondary disease was achieved by the introduction of anti-lymphocyte serum to treat the bone marrow recipients after the transplantation.

KEYWORDS

MACACUS RHESUS
BONE MARROW
TRANSPLANTS
IMMUNITY
IRRADIATION
RADIATION PROTECTION
LEUCOCYTES
ANTIGENS
ANTIBODIES
PRODUCTION
CONTROL

CONTENTS

INVESTIGATIONS ON BONE MARROW TRANSPLANTATION IN IRRADIATED ANIMALS (+)

INTRODUCTORY NOTE

For the contract period 1966 - 1967 it was proposed to concentrate on methods aimed at the selection of bone marrow donors with maximum histocompatibility with regard to the irradiated recipients. In the preceding years the suppression of acute secondary disease had been effectively achieved by the early administration of certain chemotherapeutic agents, but this treatment was not sufficient to cause long lasting control of secondary disease and stable chimaerism. It was envisaged that a reduction of the degree of histoincompatibility between the donors and the recipients would allow further improvements of the condition of the bone marrow chimaeras. These methods of selection are obviously of similar importance for the field of organ transplantation and the study in monkeys would facilitate extrapolation of the results to man.

The main approach to the problem of selection is the typing of leucocyte antigens, as is currently being pursued in man. Most of the work carried out during 1966 has been on this subject. In addition experiments have been carried out with the so called "third man test", which is based

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on the sensitization of a third party with skin grafts of the recipient, followed by grafting of skin from a number of prospective donors.

Further control of secondary disease was attempted by the use of two new methods. The first - protection of the host by specific antisera - was not reproducibly successful. The second - treatment with heterologous anti-monkey lymphocyte sera - was strikingly effective but the side-effects caused by this treatment, were found to constitute a major obstacle.

A considerable amount of preliminary and fundamental work was performed on the effects of anti lymphocyte sera in mice, using a model which initiates acute secondary disease as it occurs in monkeys and man following grafting of homologous bone marrow.

Using rodent tissues a quantitative technique for the determination of immune competent cells was employed to study the kinetics of the population of immunologically active cells in an attempt to collect more information on the development of the graft versus host reaction and of immunological tolerance. With this technique the radiosensitivity of the immune response and the speed of recovery after irradiation can be quantitatively determined.

Histocompatibility testing in Rhesus monkeys

a. Studies on the applicability of the third man test.

The third man test is a comparatively simple method to detect similarities in the transplantation antigen make up between individuals. It has been employed by Mathé for the selection of relatively compatible donors in human bone marrow transplantation. It was found that the test can also be easily performed in monkeys by grafting pieces of skin on the back. A monkey receives a skin graft from another monkey (first party or bone marrow recipient). After the graft has been rejected, skin grafts from a number of other individuals (second parties or potential bone marrow donors A, B, C) are made on the same individual (third party). The rejection pattern of the grafts A, B and C is determined by antigenic similarities between these and the recipient, in other words, the graft that is rejected most rapidly is from a donor that is probably the most similar to the first party (recipient). Only similarities, not differences, are detected by this technique. Also, the results of the test are strongly influenced by the immunogenetic composition of the third party.

Several bone marrow transplantation experiments were performed with donors and recipients selected for maximal and minimal histocompatibility. The degree of secondary disease which developed was not well correlated with the results of the third man test, which is probably due to the failure of the test to detect differences between individuals (1). The method was therefore abandoned. In a separate study using both M. rhesus and M. speciosa monkeys, it was shown that similar information can be obtained by a much simpler serological procedure based on the recognition of leucocyte antigens by multispecific iso-antisera in vitro. Some representative results are shown in Table I.

On the basis of these results a selection method for clinical use has been prepared (4) which is performed as follows. A patch of skin from the patient would be placed on a third party volunteer and this should preferably be followed by one or two leucocyte injections intradermally, to obtain a stronger serum. This serum would be the reagent in a cytotoxicity test on the leucocytes of potential donors. The leucocytes reacting strongly with the serum would be the ones having some strong transplantation antigens in common with the patient. The most eligible of such preselected donors should preferably be further screened by other histocompatibility tests - including, if desired and feasible, the actual grafting of skin on the same previously immunized third party.

b. Leucocyte antigen typing.

In collaboration with the Department of Immuno-haematology at Leyden "mono-specific" serological reagents have been produced by iso-immunisation and absorbtions, guided by the results of computer analyses of serum reactivities (11).

In this way sera were obtained which recognize two probably allelic leucocyte antigenic complexes, the composition of which is under further investigation. The importance of these two antigens (named I^a and I^b) for histocompatibility was demonstrated with skin grafting experiments. This became possible by the availability of anti-lymphocyte-serum which has a powerful immunosuppressing activity. Such immunosuppression is required to cause a sufficient spread of the range of skin graft rejection

Table I

COMPARISON OF SERUM CYTOTOXICITY AGAINST LEUCOCYTES AND HOMOGRAFT REJECTION-TIMES IN PREVIOUSLY IMMUNIZED MACACA SPECIOSA

Third party (previously immunized)	Third party serum cytotoxicity against leucocytes		Skin graft rejection-time * (days)
	Type	% toxicity	
A	1	55	6
	2	10	8
	3	10	10
	4	10	10
	5	10	10
B	1	5	10
	2	10	9
	3	90	5
	4	15	8
	5	50	5
C	1	10	10
	2	100	5
	3	60	6
	4	5	10
	5	15	10
D	1	10	10
	2	10	10
	3	100	7
	4	5	9
	5	100	5

* Skin grafts from same donor as leucocyte sample tested. Previous immunization was done by one skin graft followed 2 weeks later by a subcutaneous or intravenous injection of 200×10^6 leucocytes; third party reactivity against this donor is not included in table. Graft rejection is accelerated if it occurs before day 8

times to allow a distinction between compatible and incompatible donors.

Preliminary experiments, using ALS in monkeys, showed that incompatibility for either antigen I^a or I^b significantly reduced the survival time of a skin graft. This refinement of tissue typing in Rhesus monkeys may obviously be of major importance for successful transplantation of homologous bone marrow and of organ transplantation. Data are being collected to establish the significance of compatibility for leucocyte groups I^a and I^b for the severity of secondary disease in irradiated monkeys.

In addition the technique of kidney transplantation in the Rhesus monkey is being developed in collaboration with the Surgical Department at Leyden to permit the future evaluation of the significance of the presently identified leucocyte antigen groups for the selection of suitable kidney donors.

The identification of antigens I^a and I^b also facilitates the detection of further major leucocyte and transplantation antigens. Host/donor combinations for immunisations are now being selected to yield antisera not directed against antigens I^a and I^b. These sera are likely to lead to the serological identification of new major antigens within the near future. Obviously, recognition of new, defined, antigens makes host/donor matching according to leucocyte antigens more meaningful.

Suppression and prevention of secondary disease in primates

Following the renewed interest in the use of anti lymphocyte sera (ALS) to facilitate homotransplantation it was decided to investigate the possibilities of ALS to prevent or suppress secondary disease following homologous bone marrow transplantation. For this purpose an extensive program in mice was initiated. At the same time the production of anti-monkey lymphocyte sera and the evaluation of their capacity to prolong skin homografts was started. It soon became apparent that ALS is a most powerful agent in counteracting acute secondary disease in mice. It was found to be most effective when employed to pretreat the spleen donors but a selective inhibition of the graft versus host reaction could

also be obtained by exposing the spleen cells in vitro to ALS prior to grafting and by treating the recipients after the transplantation of homologous spleen cells. So far these experiments were performed with rabbit anti-mouse lymphocyte serum, but large batches of horse anti-mouse lymphocyte serum have now also be produced for isolation of the active γ globulin, which will be used in further studies.

In the initial part of the studies in Rhesus monkeys, Cynomolgus and Rhesus anti-lymphocyte sera were produced, later to be substituted by rabbit and horse sera. The latter were much more effective in prolonging the survival of a homograft.

With crude rabbit ALS a treatment regimen has been worked out which allows virtually permanent survival of skin homografts, without causing clinical signs of toxicity of the ALS. These results justify the conclusion that ALS is the most powerful immunosuppressive agent so far developed. Its application in experimental and clinical organ transplantation is now well under way in a number of centers, including the Rijswijk-Leyden group.

Application to the problem of secondary disease in monkeys has also yielded most encouraging results. By the administration of large doses of ALS to the irradiated bone marrow recipient after the grafting it proved possible

1. to prevent the acute phase of secondary disease as effectively as can be achieved by the early administration of cyclophosphamide
2. to prevent the later phase of secondary disease to a large extent. Unfortunately monkeys thus treated have succumbed from wide spread virus infections, identified as the cytomegaly virus.
3. to control and reverse acute secondary disease after it has become clinically manifest.

These results are summarized in Table II.

Some of these results have been published (11), and a report containing the main data of this investigation is in press (7).

It seems therefore that a method has been described to treat secondary disease effectively. In monkeys at least, the degree of immunosup-

Table II

RESULTS OF TREATMENT OF ACUTE AND DELAYED SECONDARY DISEASE IN MONKEYS WITH ALS

		Monkey nr.	ALS 1st day	ALS dose ml/day	Survival time days	Secondary disease	Compl.
	monkey	643	13	5	> 400	-	phlebitis and edema
	ALS i.v.	727	10	5	21	severe	phlebitis and edema
Early cyclo, followed by ALS	rabbit-ALS s.c.	712	13	2 - 5	23	moderate	virus
		792	16	4 - 6	24	severe	-
		704	13	5 - 10	33	slight	virus
		798	14	5 - 20	40	slight	virus
		753	11	10 - 20	33	slight	virus
No cyclo, ALS only	rabbit - ALS s.c.	781	2	2 - 10	39	moderate	virus
		805	2	5 - 15	33	slight	virus

pression necessary to achieve this causes the recipients to fall victim to a fatal virus infection. It can only be established by future investigations if and how this complication can be avoided. Obviously it is not known for certain whether a similar susceptibility to endogenous virus infections will occur in humans as a result of intensive treatment with ALS, but on the basis of close similarity in response between monkeys and man, it should be considered an extremely serious hazard.

The in vitro effect of anti-monkey LS on monkey bone marrow suspensions was disappointing, in that the selectivity of its action was insufficient for practical purposes. It was not possible to obtain reproducible elimination of the immunologically active cells with concommittent preservation of the hemopoietic cells in the suspension. This approach was therefore abandoned.

Prior treatment of the donor with doses of ALS which were capable of prolonging homograft survival were completely ineffective in decreasing the severity of the acute secondary disease. However, for the results obtained in mice it might be concluded that these doses of ALS have been too small. Additional experiments with higher doses of ALS are therefore envisaged.

Furthermore, an effective horse anti-monkey LS has been produced which will be used to produce a purified γ globulin for further investigations concerning the suppression of secondary disease and to study the occurrence of pathological effects of the prolonged treatment with ALS in monkeys.

Investigations on the quantitative determination of immune competent cells in rodent tissues

The comparative study on "cluster" and "plaque" formation by antibody forming cells in vitro has been completed. A publication entitled: "Antibody production by isolated spleen cells; a study on the cluster and plaque technique" is in preparation. From these investigations the following conclusions could be drawn:

1. Cells producing 19 s antibodies against sheep red blood cells will form clusters when incubated in a suspension together with sheep RBC.

Plaques are formed when these cells are embedded in agar and complement is added.

2. Cells producing 7 s antibodies against sheep RBC also form clusters when incubated in suspension with sheep RBC. Plaque formation in agar will however occur only when an anti-mouse globulin serum is added with the complement (indirect plaque test).
3. A large portion of the antibody forming cells produce insufficient antibody molecules to cause visible plaque formation. These cells can still be discerned with the more sensitive cluster technique.
4. About 40 per cent of the total number of cluster forming cells do not excrete antibodies although they will fix the antigen (sheep RBC) on their cell surface.

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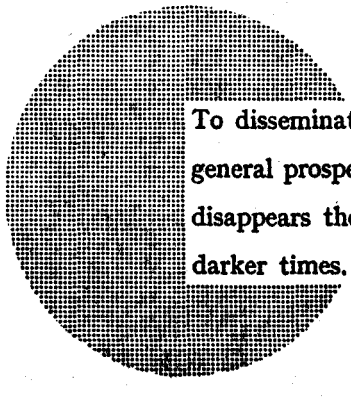

To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel

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