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**INVESTIGATIONS ON BONE MARROW
TRANSPLANTATION
IN IRRADIATED ANIMALS**

Final Report

by

H. BALNER

1971



Report prepared at the TNO
Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek
Rijswijk - Netherlands
Radiobiologie Institute
Association No. 062-66-1 BIAN

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In 1967 most of the efforts have concentrated on the procedure for the production and purification of the agent. Major progress has been made by finding that anti-human lymphocyte serum is highly immunosuppressive in chimpanzees, which enables a more rational evaluation of the agent for clinical purposes.

The studies concerning the radiosensitivity of the immune system have concentrated on the recovery process after irradiation and on the role of the macrophages in the initiation of the immune response.

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BONE MARROW
ANTI SERA
RADIOSENSITIVITY
REGENERATION
IRRADIATION
PHAGOCYTES**

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INVESTIGATIONS ON BONE MARROW TRANSPLANTATION
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Final Report

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 chimerism. It was envisaged that a reduction of the degree of histo-incompatibility between the donors and the recipients would allow further improvements of the condition of the bone marrow chimeras. These methods of selection are obviously of similar importance for the field of organ transplantation and studies in monkeys would facilitate extrapolation of the results to man.

In the period covered by the present report the identification of primate leukocyte antigens has been continued and a number of new "candidate" leukocyte antigens has been added. In addition, the study of erythrocyte groups and their significance for transplantation immunology has been initiated.

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TISSUE TYPING

a) Leukocyte antigens:

The methods to obtain "monospecific" serological reagents are based upon iso-immunization and absorptions that are guided by the results of computer analysis of serum reactivities. In this manner we obtained sera recognizing two probably allelic leukocyte antigens or antigenic complexes (closely linked antigens).

The importance of these two antigens (named Ia and Ib) for histocompatibility was demonstrated in skin grafting experiments. A powerful immune depressant was necessary to spread the range of skin graft rejection times in Rhesus monkeys sufficiently to be able to distinguish compatible from incompatible individuals. With the application of heterologous antilymphocytic sera (rabbit anti-Rhesus lymphocyte sera: RARS) we were recently able to prove that leukocyte antigens Ia and Ib are important transplantation antigens, i. o. w. that incompatibility for either antigen significantly reduces the survival time of a skin graft and, by implication, any other transplanted tissue or organ. This refinement of tissue typing in Rhesus monkeys may obviously be of great importance for homologous bone marrow transplantation after irradiation.

More or less the same methods that led to the detection of the first two antigens (Ia and Ib) have been used for identification of additional groups. Iso-antisera were made by "selected" immunizations (based on typing for antigens Ia and Ib) and these sera were tested against a multitude of leukocyte samples; repeated computer analysis of serum reactivities led to the probable identification of several new antigens. However, much additional serological work will be necessary before these specificities, tentatively called 3 - 7, can reliably be called "antigens" and before their relevance for histocompatibility will have been determined in bone marrow and skin grafting experiments.

Preliminary analysis has revealed that certain genetic associations exist concerning the distribution of these new "antigens" with each other and with antigens Ia and Ib. If confirmed, this would imply that one chromosomal area or "locus" controls the major leukocyte and histocompatibility antigens in Rhesus monkeys, as it does in mice (the H2 locus) and presumably in man (the HL system). Family studies of the inheritance of leukocyte antigens are of immense importance for this type of genetic analysis and the steady increase in the number and size of Rhesus families in our laboratory is a most welcome and advantageous development.

b) Erythrocyte antigens:

It is now generally accepted that leukocyte antigens are the main histocompatibility factors that can be identified with the presently available techniques. In man, of the known red cell antigens or conventional "blood groups", only the ABO system is considered of importance for histocompatibility. Rhesus monkeys have no equivalent of the ABO system, that is, their sera do not contain naturally occurring hemagglutinins. Previous transplantation experiments in this laboratory with pre-sensitized monkeys (subsequent recipients of skin grafts) suggested that no major transplantation antigens could be identified on red cells of Rhesus monkeys with that particular technique. Other investigators, however, have found compatibility for certain erythrocyte antigens important for the immediate "take" of kidney grafts in Rhesus monkeys (Murphy et al., Invest. Urology 3: 3, 1965).

It was felt that, parallel with the search for leukocyte antigens, the blood groups of Rhesus monkeys and their relevance for histocompatibility, particularly with regard to bone marrow transplantation, should be more closely investigated. A broad cooperation in this field with Dr. A. S. Wiener and Dr. J. Moor-Jankowski of New York has been started.

ANTI-LYMPHOCYTE SERUM (ALS)

Rabbit anti-mouse lymphocyte serum (RAMS) was found to be effective when employed in each of three different ways in reducing acute secondary disease in the mouse model;

- 1) by treating the donor mice with RAMS;
- 2) by exposing the donor spleen cells in vitro to RAMS;
- 3) by treating the recipients after the homologous spleen cells had been transplanted.

It should be recollected that positive results in the mouse model of acute secondary disease are only obtained when the agent under investigation causes a selective elimination of immunologically active cells over hemopoietic stem cells.

Rabbit anti-Rhesus lymphocyte serum (RARS) has been extensively studied:

- a) for its activity in influencing the blood picture of the normal monkey;
- b) for toxic effects in normal monkeys;
- c) for its ability to suppress the homograft reactivity against skin transplants in normal monkeys.

A treatment schedule has been worked out which allows indefinite survival of the homografts without causing clinical signs of toxicity in the recipients.

Using this information the RARS has been employed to study suppression of both acute and late secondary disease in monkeys receiving homologous bone marrow following whole body irradiation. So far preliminary results have been obtained when RARS was used to treat the monkeys after bone marrow transplantation. The effect of RARS on acute secondary disease is at least as good as that of cyclophosphamide. Responses obtained with RARS in the treatment of acute secondary disease appear to be better than have been found with any other agent investigated thus far.

After these first very encouraging results with respect to suppression of homograft reactivity and graft versus host reactivity both in rodents and primates during 1966, emphasis was placed on purification of globulin fractions and attempts to eliminate some of the undesirable

early side-effects of ALS in rodents and monkeys.

Effective suppression of acute secondary disease in monkeys with ALS has been confirmed. The lethal virus infections which have been found to complicate this treatment have been identified as adenovirus and cytomegalic inclusion body disease. The origin of these infections is being investigated. Extension of the experiments in which the bone marrow donor was pretreated with large doses of ALS has confirmed our previous preliminary observation that this is not an effective method to prevent secondary disease in monkeys.

RADIOSENSITIVITY OF THE IMMUNE SYSTEM

Techniques:

The investigations on antibody production by single spleen cells *in vitro* were continued. The number of "cluster" and "plaque" forming cells present in the spleen at different times after immunization of mice with sheep red blood cells was determined. It was found that after immunization the increase in the number of cluster and plaque forming cells followed a parallel course; however, the number of cluster forming cells was roughly 30 times higher than the number of plaque forming cells.

By using rabbit antibodies specific for either 19 s or 7 s mouse globulins the type of antibodies produced by cluster and plaque forming cells could be determined. The results obtained indicated that cluster formation by both 19 s and 7 s antibody forming cells could occur.

It was found that a certain percentage of cluster forming cells did not produce antibodies *in vitro*. Cluster formation by these cells could be ascribed to the presence of antibodies sticking to the cell surface.

Radiosensitivity of plaque forming cells:

The radiosensitivity of immune competent cells was measured in vivo by irradiating mice followed by challenge with SRBC and estimation of the number of plaque forming cells in the spleen. The values obtained agree well with those obtained by measuring other immune capacities.

The recovery of antigen sensitive cells by repopulation was studied in more detail. It could be confirmed that this recovery is a very slow process. The time interval between irradiation and antigenic challenge

is found to be of great influence on the radiosensitivity of the antibody forming system. The relation between these two parameters is being investigated at present.

The contribution of macrophages to the reaction of the immune system following radiation:

A technique was developed to study quantitatively the antibody response of cells in diffusion chambers. Only the 19 s antibodies appeared to be formed. Presently, the role of macrophages in the induction of antibody synthesis is being investigated; the preparation of macrophage fractions requires non-contaminated macrophage suspensions. When harvested from the peritoneal cavity, such suspensions nearly always contain varying numbers of lymphatic cells; the presence of which prevents a reliable interpretation of the results. Methods for the preparation of highly purified macrophages are being developed.

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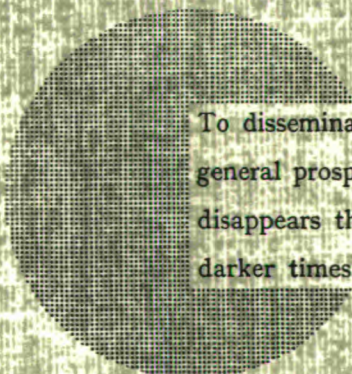
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Alfred Nobel

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