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The Transplantation Barrier of Nude Mice

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Abstract

Syngeneic memory cells can be stimulated to yield a secondary immune response after their transfer into irradiated euthymic recipients as well as into young thymusless nude mice. It is shown that nude mice older than twelve weeks of age are not permissive towards memory cell activation as it is found in non-irradiated euthymic animals. This barrier to isogeneic or congeneic cells seems to be caused by a pool of cyclophosphamide-sensitive cells. Since young nude mice could be rendered as unpermissive as older nude mice by pretreatment with either PNA-agglutinable thymus cells or nylon-wool passed spleen cells, it is suggested that an increased number of precursor T cells in older nude mice might induce this effect. Further experiments with monoclonal antibodies against the Lyt-1, Lyt-2, and L3T4 marker on T cells indicate that T-helper/inducer activity might be required to establish the «isogeneic barrier» in nude mice.

Introduction

Memory cells transferred to intact animals of the same genotype face a barrier which severely affects their capacity to function in the isogeneic or congeneic host («isogeneic barrier»). The existence of an impairment of the functional activity of transplanted immune cells has been observed by many investigators (1–4). The cellular mechanism responsible for the induction of this suppression has yet to be defined. It has been suggested that this transplantation barrier in non-irradiated animals towards *thymus-dependent* memory cells is due to a *thymus-dependent suppression* or rejection (5). But it has been shown recently that also nude mice can be non-permissive towards *thymus-independent* congeneic immune cells (6). From recent experiments, we conclude that the «isogeneic barrier» is linked to the differentiation stage of B cells in the recipient (7), since B cell-defective xid-mice are permissive towards transplanted lymphocytes.

In the present study, we investigated whether permissiveness of nude mice towards *thymus-dependent* memory cells is related to an impairment of T cell-dependent *B cells*. Our data suggest that non-permissiveness of

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nude mice is dependent on the presence of L3T4$^+$ cells which are required for enabling nude B cells to act in the «isogeneic» barrier.

Materials and Methods

Animals

All mice, derived from our own breeding stock, BALB/c, maintained by continuous brother-sister mating, were used as donor for thymus-derived cells at the age of four to five weeks or were taken as irradiated recipients at the age of eight to twelve weeks. Various aged BALB/c-nude mice from the 10th to 12th backcross generation were used for the experiments described here. The life span of these mice was about 18 weeks. Those nude mice which survived for eight months were bred and maintained under germ-free conditions.

Antigen and immunization

TNP-haptenated sheep red blood cells (TNP-SRBC) were prepared as described by RITTENBERG and PRATT (8). The mice were immunized intraperitoneally (i.p.) with a single dose of 3×10^8 TNP-SRBC.

Memory cells

BALB/c mice were immunized with 3×10^8 TNP-SRBC. Four to six weeks later, spleen cells from these mice were used in an adoptive transfer and stimulated again with antigen to activate memory cells in order to give a secondary response in the host.

Treatment with cyclophosphamide (Cy)

Cyclophosphamide (ASTA, Brackwede, F.R.G.) was applied i.p. at a single dose of 20 mg/kg body weight 24 h before the administration of memory cells and antigen.

Antisera

Monoclonal anti-Thy-1.2, anti-Lyt 1.2, and anti-Lyt-2.2 were obtained from New England Nuclear (Boston, MA, U.S.A.). Depletion of cells bearing the appropriate marker was achieved by incubating twice 1×10^7 spleen cells/ml with specific monoclonal antibodies adequately diluted in Hanks' balanced salt solution, washed and treated with rabbit complement for 30 min at 37 °C. Complement was selected for low cytotoxicity towards mouse cells. Monoclonal anti-L3T4 antibodies (GK 1.5) were derived from hybridomas as described by DIALYNAS et al. (9).

Differential agglutination of mouse thymocytes by peanut-agglutinin (PNA)

Separation of agglutinated (PNA$^+$) and non-agglutinated (PNA$^-$) thymus cells was carried out as described by REISNER et al. (10). Cells were dissociated with 0.3 M D-galactose. PNA was purchased from IBF Pharmaindustrie, Villeneuve la Garenne, France. The agglutinated subpopulation represents 80–90 % of the total number of thymocytes from four-week-old donors.

Separation of lymphocytes

T and B lymphocytes were separated from whole spleen cell suspensions by passage through a nylon-wool column as described by JULIUS et al. (11). Fluorescence studies showed that nylon-wool-passed cells contained 98 % Thy-1-positive spleen cells. The nylon-wool adherent fraction included 95 % Thy-1-negative cells.

Hemagglutination assay

Serum antibody titer against the hapten TNP was determined 10 days after immunization by hemagglutination of TNP-coated horse red blood cells in polystyrene micro-titer plates using Takatsy loops. Two-fold serial dilutions were used. The hemagglutination titer is referred to as $\log_2$ dilution of the highest dilution yielding positive hemagglutination. All sera were treated with 2-mercaptoethanol for thirty minutes at 37 °C.

Irradiation

Irradiation of mice was performed with a Siemens Stabilipan (Siemens, Stuttgart, F.R.G.), operating at 250 kV, 15 mA with 0.5 mm Cu and 1 mm Al filters. Mice received a dose of 650 R.

Results

Age-dependence of the isogeneic barrier in nude mice

The adoptive immunity in BALB/c nude mice of different ages was investigated and compared to the immune response in non-irradiated normal BALB/c recipients of various ages. As positive control recipients, we used lethally irradiated (650 R) BALB/c mice. The animals were injected with a dose of 8×10^6 spleen cells from immunized mice and 3×10^8 TNP-haptenated sheep erythrocytes (TNP-SRBC). The data given in Figure 1a

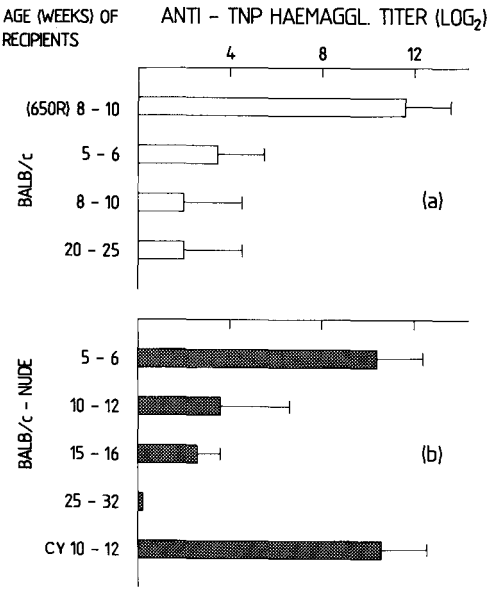

Figure 1. Age-dependence of the isogenic barrier in nude mice. Mice of different age received 8×10^6 memory cells and 3×10^8 TNP-SRBC. Ten days later, the antigen-specific secondary response of donor cells to TNP was determined by hemagglutination assay in the presence of 2-mercaptoethanol. Four mice were used for each group, and the results are presented as the mean values of three experiments. The vertical bars represent standard deviations.

demonstrate the lack of memory activation in non-irradiated, normal BALB/c mice independent of their age. The secondary immune response (mercaptoethanol-resistant) of five, ten and twenty-week-old mice is significantly lowered in comparison to the positive control of irradiated animals.

In contrast to this result, the ability of nude mice to permit an adoptive immune response decreases strikingly with age: after injection of congeneic memory cells, the secondary immune response of young nude mice (five weeks) is not distinguishable from that of lethally irradiated hosts. On the other hand, older nude mice were less permissive towards congeneic memory cells. They show a transplantation barrier towards memory cells, similar to non-irradiated euthymic animals (Figure 1b).

In an additional experiment, older nude mice were injected with a low dose of cyclophosphamide (20 mg/kg) prior to the injection of memory cells and antigen. From the data given in Figure 1b, it is evident that older nude mice treated with cyclophosphamide are as permissive towards transplanted cells as lethally irradiated normal mice.

T cell-dependence of the transplantation barrier towards T cell-dependent memory cells

Since it had been shown that the isogeneic barrier towards thymus-dependent memory cells is lost in nude mice (5), we asked whether permissiveness of thymus-deficient mice is based on the absence of specialized T cells. In order to investigate this question, a group of young nude mice (4–5 weeks old) was treated with nylon-wool non-adherent spleen cells from normal mice. To permit homing of these cells in the nude recipients, the administration of congeneic memory cells followed two days later. The results of these experiments are shown in Figure 2: the isogeneic

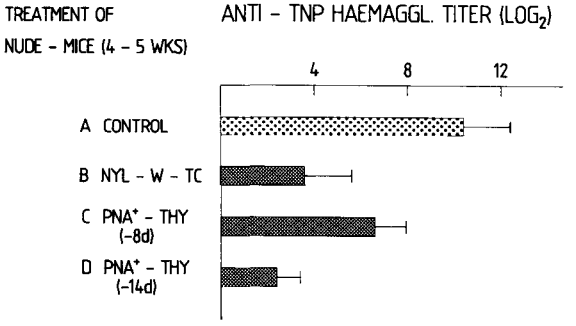

Figure 2. T cell-dependence of the transplantation barrier towards T cell-dependent memory cells. Four- to five-week-old nude mice received either 5×10^6 nylon-wool-nonadherent spleen cells or PNA-agglutinable thymocytes from normal mice. Two, eight or fourteen days later, these animals were injected with 8×10^6 memory cells and antigen. Ten days thereafter, the antibody titer was determined by hemagglutination assay in the presence of 2-mercaptoethanol.

barrier can be introduced to young nude mice by inoculation of mature nylon-wool non-adherent spleen cells (B).

Since it is known that older nude mice possess Thy-1^+ precursor cells in contrast to younger animals (12), we investigated whether immature precursor T cells from the thymus can also restore the isogeneic barrier in young nude mice. Therefore, a second group of four-week-old nude mice received an injection of PNA-agglutinable thymus cells (C) prior to the introduction of memory cells and antigen. The data of these experiments are also summarized in Figure 2. In comparison to non-treated mice (A), the ability of young nude mice pretreated with PNA^+ cells to mount a secondary response was significantly reduced. In contrast to nylon-wool non-adherent spleen cells, the PNA-agglutinable cells required a longer homing period of fourteen days to display an intensified suppressive activity (D). These data indicate that precursor T cells developing from an immature stage can induce a transplantation barrier in nude mice.

Non-permissiveness of nude mice towards T cell-dependent memory cells is dependent on the presence of Lyt-1^+ lymphocytes with L3T4 determinants

In order to examine which T cell population is required for the induction of the transplantation barrier in older nude mice, spleen cells from normal mice were treated with monoclonal anti-Lyt-1, anti-Lyt-2 or anti-L3T4 antibodies in the presence of complement and injected into young nude mice two days prior to transplantation of memory cells. The results of these experiments are depicted in Figure 3. Inoculation of untreated, syngeneic

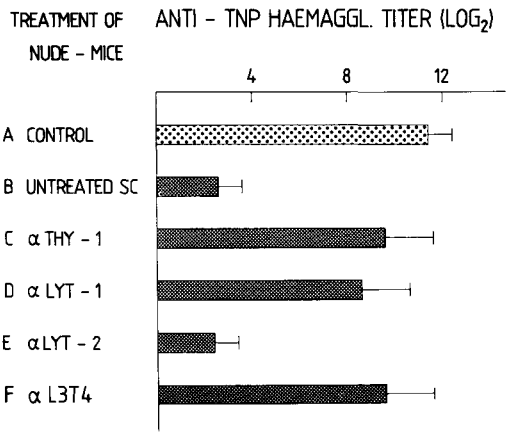

Figure 3. T cell subpopulations required for the development of the transplantation barrier in nude mice. Spleen cells from normal BALB/c mice (1.5×10^7), treated either with monoclonal anti-Thy-1, anti-Lyt-1, anti-Lyt-2 or anti-L3T4 antibodies, were injected into young nude mice two days prior to transplantation of memory cells and antigen. The secondary response (ME-resistant) of donor cells was examined 10 days later.

spleen cells into young nude mice two days prior to transplantation of memory cells leads to a transplantation barrier in these animals (B). After elimination of T cells by monoclonal anti-Thy-1.2 antibodies, the ability of BALB/c spleen cells to introduce a barrier in young nude mice is lost (C). Also treatment with monoclonal anti-Lyt-1 antibodies leads to an impairment of these cells (D). On the other hand, depletion of lymphocytes carrying the Lyt-2 marker does not affect the capability of splenic T cells to introduce a barrier into young mice (E), whereas treatment of spleen cells with monoclonal anti-L3T4 antibodies and complement leads to permissiveness of nude mice towards transplanted immune cells (F). From these data, we suggest that L3T4$^+$ cells rather than Lyt-2$^+$ T cells play a role.

Discussion

Congenitally athymic nude mice are deficient in T cell function (13) as indicated in graft-versus-host-reaction (14) and skin graft rejection (15).

Immunofluorescence studies demonstrated, on the other hand, the presence of Thy-1-positive T cell precursors in the bone marrow and the peripheral lymphoid tissues of nude mice (16, 17). These investigations have led to the view that athymic nude mice possess immature precursors of functional T cells whose maturation is blocked in the absence of thymic influence. Thymic products can mediate a signal for further differentiation of nude T cell precursors (18). It was also shown that spleen and lymph nodes from nude mice older than six months of age contain significant numbers of cells expressing the Thy-1 and Lyt-1 antigen (12). Thus, older mice possess lymphocytes expressing surface markers characteristic for differentiated T cells, although they lack a functional thymus.

Since transplantation of immune cells can be achieved successfully in irradiated or nude mice but not in normal isogeneic recipients (2, 5), we have investigated whether permissiveness of congeneic nude mice towards T cell-dependent memory cells is age-dependent. Our data indicate that in contrast to euthymic animals, only young nude mice (five to six weeks old) were as permissive towards congeneic memory cells as lethally irradiated normal recipients. But nude mice more than twelve weeks of age did not permit an adoptive response (Fig. 1). This effect could be abolished by pretreatment of the recipients with cyclophosphamide. The introduction of nylon-wool non-adherent spleen cells, as well as the administration of PNA-agglutinable, immature thymus cells, rendered young mice as unpermissive towards transplanted memory cells as older animals (Fig. 2). This suggests that precursor T cells found in older mice (12) might exert a similar effect. The fact that the injected PNA-agglutinable thymocytes required a longer period for displaying an intensified activity (Fig. 2), implies that these precursor cells have to undergo further differentiation or that contaminating mature T cells have to be accumulated.

Maturation of T precursor cells from bone marrow, embryonic liver and the spleen of *nude mice* has been demonstrated already *in vitro* (18, 19). Also the induction of Thy-1-positive, cyclophosphamide-sensitive suppressor cells in nude mice has been reported (20). A study by RANGES et al. (21) describes the maturation of TL^+ cells into Lyt-1-subsets of nude mice older than ten weeks.

Our experiments with monoclonal antibodies against the Lyt-1, Lyt-2, and L3T4 marker on T cells indicate that T-helper/inducer activity might be required to establish the «isogeneic barrier» in nude mice (Fig. 3). From our data, it is tempting to speculate that there is an association between the increased occurrence of T-lineage cells and the development of a transplantation barrier in older nude mice. Since experiments with nude mice also demonstrated the appearance of suppressor cell activity (20, 22, 23), we attempted to identify suggested suppressor cells in older nude mice by transferring them into irradiated hosts prior to transplantation of stimulated lymphocytes. However, we were unable to detect any suppressive effect of spleen- or lymph node-cells taken from nude mice, demonstrating a transplantation barrier (H. MOLL and R. BÖSING-SCHNEIDER, unpublished results).

From recent data obtained from experiments with (CBA/N × BALB/c)F_1 mice, we have concluded that mature B cells are the agent of the «isogeneic barrier» effect (7), since B cell-defective mice are permissive towards transplanted immune cells. Therefore, we suggest that T cell-dependence of the transplantation barrier in nude mice reflects a $L3T4^+$ cell-dependent activity of *B cells* leading to resistance towards transplanted lymphocytes. Experiments with xid-mice also imply that idiotype-anti-idiotype reactions displayed by B cells are a prerequisite of the transplantation barrier towards T cell-independent lymphocytes (7, 24, 25).

Other data from our group demonstrate that the barrier in nude mice does not result in elimination of transferred donor cells but leads to persistence in the nude host (6, 26).

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