INDUCTION OF IgG IN YOUNG NUDE MICE BY LIPID A OR THYMUS GRAFTS*

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Lipopolysaccharide (LPS)[1] from gram-negative bacteria, or the lipid A component isolated from LPS, have a potent stimulatory effect on B lymphocytes. In vitro, they induce both cell proliferation and IgM secretion (reviewed in references 1–3). In vivo, we have demonstrated a pronounced effect of lipid A administered to mice during the first few days of life: serum IgG rose rapidly to levels 10- to 50-fold higher than in untreated controls of the same age (4). By contrast, adult animals did not show a clear response to LPS or lipid A; the ability to respond to lipid A with IgG production is lost between 14 and 20 days of age (unpublished results). The dramatic response of new-born mice to lipid A is of interest in terms of the ontogenetic development of the Ig-producing limb of the immune system. We therefore studied the early Ig production in congenitally thymusless ("nude") mice and their response to lipid A and thymus grafts. The experiments to be presented were also prompted by reports showing that the limiting factor governing the capacity of young mice to give a humoral immune response could be the presence or maturity of thymus-dependent "helper" cells (5, 6).

A number of studies in vitro are concerned with the question whether or not T cells are participants in the stimulation of B cells by LPS or lipid A (7–9). The interpretation of the results is complicated by the finding that the nude mouse does possess some θ-positive cells (10), and that, in fact, LPS itself can induce precursor cells to express T-cell-specific antigens (11).

In the design of the present experiments [as with those reported previously (4)] we had to take cognizance of the fact, that young animals bear high serum levels of maternal IgG. To measure selectively the autochthonous production of IgG by the young, we had to use allotypically heterozygous offspring, and assay only the Ig bearing the paternal allotype.

The experiments show that lipid A can induce accelerated IgG production in young nudes, albeit not to the same degree as in normal young mice. IgG production was also dramatically stimulated by nearly congeneic thymus grafts; and the effects of lipid A and thymus grafts were additive and possibly cooperative.

Materials and Methods

Mice. The following mice were bred in our laboratory: BALB/c-AnNIcr; BALB/c-nu, a backcross-line developed in Konstanz by B. M. Kindred, which carries the nude (athymic) factor on the

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[1] *Abbreviation used in this paper:* LPS, lipopolysaccharide.

BALB/c-AnNIcr background; C57BL/6-JNIr; C57BL/6-Ig^a, a new backcross line carrying the Ig^a allotype (from BALB/c) on the C57BL/6 genome of our stock; and the Ig$^{a/a}$ homozygous line was derived from the ninth backcross generation.

BALB/c-nu/+ females from the 10th to 11th backcross generation were used as mothers of the offspring in the experiments. The C57BL-nu/nu mice, serving as fathers, were from Bomholtgard Laboratories, Ry, Denmark; they were derived from the fourth backcross generation of nude (from NMRI-nu) to C57BL, and were found to carry the C57BL allotype b; for simplicity they will be called C57BL-nu, although they carry only 97% of the C57BL genome. Young mice to be tested for IgG levels were bled by puncture of the retroorbital sinus every 3–4 days, starting 12–13 days after birth.

Thymus Graft. Thymus organs were obtained from 4-day old (BALB/c × C57BL-Ig^a)F$_1$ donor mice. A whole thymus was inserted under the dorsal skin of each nude recipient through a Pasteur pipette; a subcutaneous canal had before been bored using a heavy gauge canula.

Lipid A. Lipid A was obtained by acid hydrolysis (12) of phenol water-extracted LPS (13). 1 mg lipid A was dissolved in 1 ml distilled water by addition of 0.5 μl triethylamin (14).

Antisera. Anti-sheep red blood cell (SRBC) antiserum of allotype b, and anti-allotype b antiserum reacting with IgG2a^b, were used as described (4).

Assay for IgG of b allotype. The assay system has been modified since the previous report (4). Previously, the quantity of IgG^b in a serum was measured by its capacity to inhibit the facilitation of complement-dependent hemolysis by anti-allotype b antiserum. Now we used as the basic assay system the ability of anti-allotype serum to augment the hemagglutination of SRBC (15); these SRBC had been presensitized with mouse anti-SRBC (IgG) bearing the allotype b, in subagglutinating doses. The hemagglutination end point will then depend on the concentration of anti-allotype antiserum. Free IgG of allotype b will absorb anti-allotype antibodies and consequently inhibit hemagglutination. The degree of inhibition, at fixed amounts of anti-allotype, is proportional to the concentration of IgG. C57BL normal serum inhibits hemagglutination to a dilution of 1/5,120–1/10,240. Supposing the concentration of IgG2a^b in normal serum to be between 0, 3, and 5 mg/ml, the test could detect 0 and 6–10 μg IgG2a^b/ml serum. Thus it is about fourfold less sensitive than the hemolysis assay used previously (4). However, since the present technique employs simple twofold dilution steps and readings of hemagglutination end points, using Takatsy microtiter equipment, it is considerably faster to perform.

The SRBC were sensitized with 2-mercaptoethanol-treated C57BL-anti-SRBC antiserum (allotype b). The serum to be assayed was serially diluted in anti-allotype b antiserum of a concentration fourfold that of the hemagglutination end point. The sera were allowed to react for 30 min at room temperature. Presensitized SRBC were then added and the hemagglutination inhibition titers read.

Results

Ontogeny of IgG Production in Nude Mice. Young thymusless (nu/nu) mice, derived from matings of BALB/c-nu/+ females and C57BL-nu/nu males do not show measurable amounts of IgG2a^b (paternal allotype) in their serum before the age of 4 wk (Fig. 1). They reach adult levels between 6 and 8 wk. Thus the kinetics of IgG production was not noticeably different in nude and normal mice (Fig. 2). It should be noted, however, that BALB/c-nu/nu mice display a considerably wider range of adult levels of IgG than normal BALB/c mice. The IgG levels in nudes varied between 0.1 and 10 times the normal average amount.

Effect of Lipid A. As we found previously (4), in normal mice lipid A was most effective in stimulating IgG production when injected 7 days after birth. The IgG2a^b serum levels of (BALB/c × C57BL)F$_1$ mice, treated with lipid A (85 μg/mouse) 7 days after birth (or left untreated) are shown in Fig. 2. A dramatic rise in IgG concentration occured after lipid A, as reported previously (4): levels 45-fold higher than in uninjected controls were reached by 15 days of age.

In nude mice a single injection of lipid A on day 7 of life, induced IgG2a^b

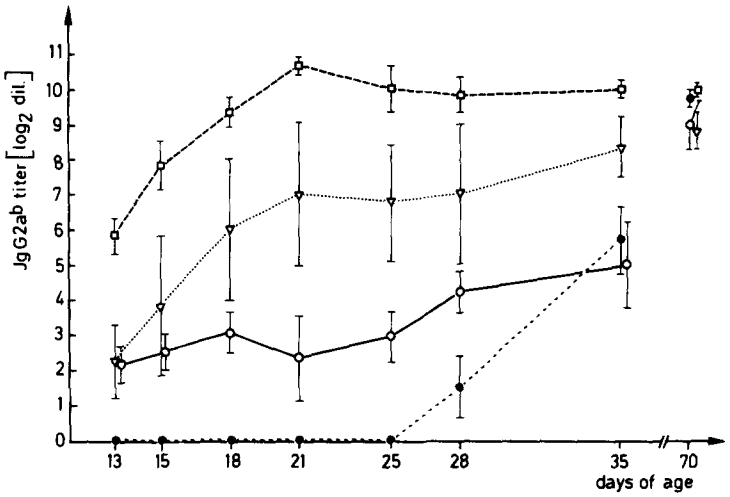

FIG. 1. IgG2a^b titers (parternal allotype) of nude F_1 (BALB/c-nu/+ × C57BL-nu/nu) mice between 13 and 70 days of age. Treatment of mice was: (●), no treatment; (○), 85 μg lipid A injected at 7 days after birth; (▽), 4-day-old thymus grafted at 4 days after birth; and (□), 4-day-old thymus grafted at 4 days and lipid A injected at 7 days after birth. The bars in the graph represent the standard deviation of the mean of four to eight mice. The initial dilution of the test sera was $^1/_{10}$ corresponding to a titer of 0 ($\log_2$ dilution [dil.]) on the ordinate.

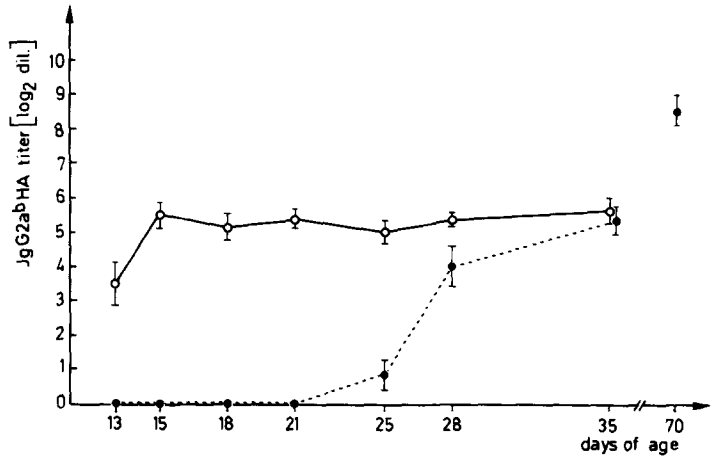

FIG. 2. IgG2a^b titers of normal F_1 (BALB/c × C57BL) mice between 13 and 70 days of age. (●), control mice untreated; (○), mice injected with 85 μg lipid A 7 days after birth. The bars in the graph represent the standard deviation of the mean of four mice. The initial dilution of the test sera was $^1/_{10}$, corresponding to a titer of 0 ($\log_2$ dilution [dil.]) on the ordinate.

production in a similar manner as in normal animals (Fig. 1). The stimulation factor, however, was only six- to eightfold and thus considerably lower than in their normal counterparts.

The Effect of Thymus Grafts. 4-day-old nudes (a/b allotype) were grafted with thymus organs from 4-day-old (BALB/c × C57BL-Ig^a)F_1 mice (see Materi-

als and Methods), and their subsequent production of IgG, allotype-b, was monitored. (The thymus graft, homozygous with respect to allotype a, thus cannot itself contribute to the production of allotype b immunoglobulin.) The results are shown in Fig. 1. Between 13 and 21 days of age there was a dramatic increase of IgG2a^b concentration; levels were up to 128-fold higher than in controls without thymus graft.

When nudes were injected with lipid A on day 7, in addition to having received a thymus graft on day 4, IgG levels again showed an increase between days 13 and 21; the actual concentrations were 8- to 16-fold higher than in recipients of a thymus graft not injected with lipid A. (Fig. 1). Doubly treated animals (thymus and lipid A) reached IgG concentrations typical of adult animals by 3 wk of age and held these levels for the ensuing 7 wk. It is apparent from Fig. 1 that treatment with thymus and lipid A leads to much smaller fluctuations of titers than treatments with thymus or lipid A alone; a suggestion perhaps that double treatment activates the B-cell compartment of the developing animal to its natural limit.

Discussion

Quite unexpected, under the generally held view of immunodeficiency in thymusless mice, was the finding that congenitally thymusless "nude" mice synthesize immunoglobulin (IgG) at a nearly normal rate during postnatal ontogeny: adult levels of IgG are reached at about the same age as in normal congeneic animals. It is striking, however, that adult steady state levels of IgG vary much more widely in adult nudes than in normal mice. This points to a greatly weakened regulatory control over immunoglobulin concentrations in mice, lacking a thymus.

Since we had previously found (4) that administration of killed typhoid bacteria, or lipid A extracted from Salmonella LPS, exerted a potent stimulatory influence on the IgG production of normal mice during the early postnatal period, it appeared of interest to study the effect of these agents in congeneic thymusless nude mice.

To perform this experiment in an in vivo situation appeared useful also in view of conflicting results concerning the effect of thymus-dependent cells on the stimulation of B cells by LPS in vitro. DNA synthesis and IgM production could be stimulated in spleen cultures from nude mice (7), but not in cultures of Peyer's patch, which are devoid of adherent cells (8). A helper role of T cells for the adjuvants effect of LPS in an antigen-specific response, but not for the polyclonal stimulation, has been reported (9). Forbes et al. (16) demonstrated a synergistic effect of LPS and mitomycin-treated allogeneic cells on the DNA synthesis of thymocytes.

Our data demonstrate a clear stimulatory effect of lipid A on the developing immune system of young nude mice. Lipid A-treated nudes had IgG levels, at 15 days of age, which were reached by the controls only 2½ wk later. However, the stimulation factor was only 6- to 8-fold in nudes as compared to about 45-fold in normal mice. Our data show that the presence of a thymus is not a prerequisite for the ontogenic development of IgG nor for its precocious stimulation by the mitogen LPS.

The possible influence of a thymus graft on the development of IgG production in newborn nude mice seemed of interest. Fig. 1 shows that a thymus graft, given at 4 days of age, causes a dramatic acceleration of IgG production: 1/4 of adult concentrations are reached already at 3 wks of age; that is, 2 1/2 wk earlier than in nudes not grafted or in normal mice. The grafted thymus must exert a profound proliferative and differentiating influence on the IgG-producing (B cell) limb of the developing immune system.

However, the following should be pointed out: The newborn nude mice used were from crosses between BALB/c-nu/+ ♀ and C57BL-nu/nu ♂. The grafted thymus tissue was from (BALB/c × C57BL-Ig^a)F_1 hybrids. The purpose of this combination was to exclude allogeneic differences between graft and host as far as possible and at the same time to be able to assay the performance of the newborn nude's IgG-producing system by virtue of the IgG-b allotype uniquely contributed by the C75BL-nu/nu father.

These C57BL-nu/nu males (which were kindly provided by the Bomholtgard Laboratories) were derived from the fourth backcross generation of the nude gene into the C57BL genome. It cannot, therefore, be excluded that parts of the original NMRI-genome, with which the *nu* locus had been associated before, was still present in our progeny and that associated with it were transplantation antigens recognized as foreign by the thymus graft. Such a situation could have provoked an allogeneic stimulation (graft-vs.-host reaction), resulting in the accelerated IgG production observed. In fact, a graft-vs.-host reaction of traditional type (P → F_1) does result in IgG stimulation (C. Kolb, unpublished observations). The issue, whether a true congeneic stimulation by thymus tissue has been measured in our experiments, or whether the observed effects were due to residual allogeneic differences between donor and host, has to be resolved in further experiments.

The capacity of the immune system of the newborn nude mouse to be stimulated to accelerated IgG production was exhausted neither by lipid A nor by thymus grafts. When both were given together, IgG production exceeded that observed when either agent was applied alone. In fact, IgG2a^b levels after combined application were two- to three-fold higher than could be accounted for by an additive effect. This result suggests a cooperation between the stimulatory activity of thymocytes and the mitogenic effect of lipid A.

Summary

Postnatal serum concentrations of IgG2a of paternal allotype, measured in congenitally thymusless nude mice, increase with kinetics and titers comparable to their normal congeneic counterparts. Lipid A, the mitogenic part of LPS, stimulates IgG synthesis in nude mice when it is given 7 days after birth. IgG concentrations at 15 days of age are 6- to 8-fold higher than in untreated control nudes; this is considerably lower, however, than in normal mice, which show up to 45-fold higher IgG2a^b levels after lipid A treatment.

A thymus graft from nearly congeneic donors of the same age, transplanted at 4 days after birth, also stimulates long-lasting IgG synthesis in the nude recipients. If the grafted nudes are injected with lipid A 3 days later, IgG synthesis is further stimulated 8- to 16-fold.

The data are discussed in relation to the thymus dependency of IgG production and the conditions for lipid A stimulation.

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