STUDIES ON RABBIT LYMPHOCYTES IN VITRO

IV. BLAST TRANSFORMATION OF THE LYMPHOCYTES FROM NEWBORN RABBITS INDUCED BY ANTIALLOTYPE SERUM TO A PATERNAL IgG ALLOTYPE NOT PRESENT IN THE SERUM OF THE LYMPHOCYTE DONORS*

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Six different genetically controlled antigenic determinants of rabbit IgG have been well defined, and have been given the name allotypes. Of the six allotypes, As1, As2, and As3 are controlled by one chromosomal locus “a”, and are located on the H chain of rabbit IgG; allotypes As4, As5, and As6 are controlled by a second chromosomal locus “b”, and are generally thought to be located on the L chain of rabbit IgG (1-3).

Rabbit lymphocytes obtained from a donor with a given IgG allotype may be stimulated to undergo blast transformation and to synthesize DNA if cultured in vitro in the presence of the appropriate antiallotype serum (4). This transformation only occurs when the donor cells are obtained from a rabbit having a given IgG allotype, and these cells are cultured in the presence of an antiserum prepared against the given allotype. Antisera to all six well defined rabbit IgG allotypes may initiate transformation of the appropriate cells (5).

The identification of the allotypic specificity of lymphocytes by lymphoblast transformation appears to be inherent in the cells and not dependent upon the presence of free IgG or of IgG picked up by the cells from the environment. Such a conclusion is based on the finding that the capability of donor lymphocytes to transform was not significantly altered by preincubation for up to 16 hours with serum that did not contain the appropriate allotype (4). This experimental result does not rule out the possibility that environmental IgG may be irreversibly or very strongly bound on or in the lymphocyte, and therefore, the IgG content of the lymphocyte not significantly alterable under the conditions of the preincubation experiment.

In order to investigate more thoroughly the possibility that allotypic specificity might be conferred on rabbit lymphocytes via environmental IgG, advan-

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RABBIT LYMPHOCYTES IN VITRO. IV

tage was taken of the observations of Dray (6), that the serum IgG content and therefore allotypic specificity of the newborn rabbit is supplied by the mother via placental transfer. Thus, the IgG allotype of a newborn rabbit will be phenotypically identical with its mother even though its genotype may be different. Accordingly, if As4 homozygous does are mated with As5 homozygote bucks, the resulting offspring will be heterozygotes, As4,5. However, for up to 5 weeks of age, only IgG of allotype As4 transferred from the mother will be detectable in the newborn. The purpose of the experiments described in the present report

TABLE I
Effect of Different Sera on the Induction of Blast Transformation in Cultures of Lymphocytes Obtained from Rabbits at 3 Weeks and at 2 Months of Age

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Serum allotype (3 weeks)</th>
<th>Serum allotype (3 months)</th>
<th>Lymphocyte reactivity at 3 weeks (per cent blasts)</th>
<th>Lymphocyte reactivity at 2 months (per cent blasts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal serum</td>
<td>Anti-As1</td>
<td>Anti-As2</td>
<td>Anti-As3</td>
</tr>
<tr>
<td>59-53</td>
<td>As1,3,4</td>
<td>As1,3,4,5</td>
<td>&lt;1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(568)‡</td>
<td>(2530)</td>
</tr>
<tr>
<td>59-54</td>
<td>As1,3,4</td>
<td>As3,4,5</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2530)</td>
<td>(1500)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;1</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(12,290)</td>
<td>(2900)</td>
</tr>
</tbody>
</table>

* Allotype of maternal IgG.
‡ C14-thymidine uptake (counts/10 minutes) in parentheses.

was to test the ability of the peripheral lymphocytes from such newborn rabbits to be transformed when cultured in the presence of antiserum to allotypic determinants controlled by both maternal and paternal chromosomes.

Material and Methods

The IgG allotypes of normal healthy adult rabbits were determined by specific reference antisera (7). Female homozygous at the “b” locus for As4 or As6 were mated with males homozygous at the “b” locus for As5. Two matings: (a) As1, 3, 4 female X As1, 3, 5 male, and (b) As1, 3, 6 female X As1, 5 male, were productive of litters. At the age of 3 weeks two offspring from each of the above matings were bled by heart puncture, the peripheral lymphocytes separated, and cultured in vitro as described previously (4), and the allotype of the serum
IgG determined. At the age of 4 weeks the remaining animals in the first litter were also tested. The lymphocyte cultures each contained $1 \times 10^6$ lymphocytes. Microscopic examination of Jenner-Giemsa-stained smears obtained after 48 hours was performed and the degree of "blast" transformation recorded. Because of the small number of lymphocytes in each culture no attempt was made to measure DNA synthesis as had been done in previous experiments (4, 5). The lymphocytes of each animal were cultured with autologous serum, with antisera to the maternal allotype (As4 or As6), with antisera to the paternal allotype (As5), with antisera to the allotype controlled by the "b" locus not present in either parent (As4 or As6), and with antisera to As1 controlled by the "a" locus. At the age of 2 months lymphocytes were again obtained from each animal by ear vein bleeding and retested. Since a

**TABLE II**

**Effect of Different Sera on the Induction of Blast Transformation in Cultures of Lymphocytes Obtained from Rabbits at 4 Weeks and at 2 Months of Age**

<table>
<thead>
<tr>
<th>Parentage $As1,3,4^a \times As1,3,5^a$</th>
<th>Serum allotype (3 weeks)</th>
<th>Serum allo-</th>
<th>Lymphocyte reactivity at 4 weeks (per cent blasts)</th>
<th>Lymphocyte reactivity at 2 months (per cent blasts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>type (3 months)</td>
<td>Normal Serum</td>
<td>Anti-As1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59-49 $As1,3,4$</td>
<td>As1,3,4,5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59-50 $As1,3,4$</td>
<td>As1,3,4,5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59-51 $As1,3,4$</td>
<td>As3,4,5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>59-52 $As1,3,4$</td>
<td>As3,4,5</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Allotype of maternal IgG.

† C14-thymidine uptake (counts/10 minutes) given in parentheses.

greater number of lymphocytes was available at this age, each culture was made up with approximately $5 \times 10^6$ lymphocytes and the rate of DNA synthesis was determined as previously described (4). At 3 months of age each animal was again bled, the serum obtained, and the allotype of the serum IgG determined.

**RESULTS**

The reactivity of the lymphocytes from rabbits tested at 3 weeks and 2 months of age is given in Table I, and the reactivity of the lymphocytes from the rabbits tested at 4 weeks and 2 months of age in Table II. The serum IgG allotype of each animal when tested at 3 months of age is also shown. There was no essential difference between the per cent blast transformation induced with antisera to the maternal allotype controlled at the "b" locus and that induced
with antisera to the paternal allotype controlled at the “b” locus. Antisera directed against allotypic determinants not present in the genotype (determined by testing for allotypes in the sera of each rabbit used at age 3 months) did not induce any significant transformation.

The specificity of antiallotype induced blast transformation is shown in particular for the progeny resulting from the As1,3,4 X As1,3,5 mating that did not contain As1 (59–54, Table I; 59–51 and 59–52 Table II). Since both parents were heterozygous at the “a” locus for As1, the presence or absence of this determinant in the genotype of any given newborn could not be accurately predicted, and could not be adequately detected using the usual gel diffusion test of serum until 3 months of age. However, the absence of significant blast transformation of the lymphocytes obtained from these newborn rabbits, when cultured in the presence of anti-As1 serum, correctly indicated the absence of the As1 determinant as early as 3 weeks of age.

DISCUSSION

Blast transformation may be induced in lymphocyte cultures derived from heterozygote newborn rabbits by antiallotype sera directed against either maternal or paternal IgG allotypes at an age when only the maternal allotypic specificity can be detected in the serum of the newborn rabbits. Until about 5 weeks of age the IgG present in the serum of a newborn rabbit is that which was supplied by the mother via placental transfer (4). Thus, in the present experiments, when a doe homozygous for As4 is mated to a buck homozygous for As5, all of the offspring resulting from such a mating are heterozygous As4,5; yet the only IgG allotype detectable in these newborn As4,5 rabbits for the first few weeks of age is the As4 supplied by the mother. These newborn rabbits are genotype As4,5, but phenotype As4 in regard to serum IgG. However, blast transformation of lymphocytes from these animals at 3 and 4 weeks of age in vitro can be induced with both anti-As4 and anti-As5 sera. Therefore, the true genotype of these newborn rabbits can be identified in the absence of detectable amounts of one of the allotypes in serum IgG.

These experimental findings illustrate two important points: first, it essentially rules out the possibility that allotypic specificity of peripheral lymphocytes as determined by the allotypic blast transformation system is due to serum IgG picked up or bound to the lymphocytes from the environment; and second, since allotypic blast transformation must require a reaction of some kind between antiallotype antibodies and allotypic determinants (4), and since it is extremely unlikely that allotypic determinants could be supplied by a material other than the appropriate IgG, the lymphocytes of newborn rabbits must manufacture at least some IgG molecules or IgG chains (5). However, there is the remote possibility that the lymphocytes with the potential to transform in the presence of antiallotype serum could have received IgG from other IgG-
producing cells (i.e., plasma cells). Against this possibility is the demonstration by Thorbecke (8) that there are very few plasma cells in normal newborn rabbits and little or no evidence of IgG synthesis except in the lymphoid tissues of the intestinal tract. On the other hand, the lymphoid tissues of immature rabbits will respond to antigenic challenge (9), and spleen cells from newborn rabbits treated with antigen in vitro and transferred to adult hosts will produce good antibody titres (10). Thus, the evidence favours the potential IgG-forming capacity of the lymphoid tissues of immature rabbits under certain experimental conditions.

The induction of blast transformation in vitro by antisera may be an extremely sensitive method of detecting the potential of lymphocytes to synthesize a given protein. We have been unable to detect IgG in the peripheral lymphocytes obtained from normal rabbits by specific fluorescein-tagged anti-IgG, by I181-labelled anti-IgG, or by in vitro incorporation of labelled amino acids into IgG (11). However, the synthesis of IgG by the peripheral blood lymphocytes of hyperimmunized rabbits has been demonstrated (12), indicating that under certain conditions IgG formation by rabbit peripheral blood cells does occur. Since the basic mechanism of the induction of blast transformation is as yet unclear, any conclusions as to just what antiallotype antisera may recognize in the "stimulatable" lymphocytes are only speculative. However it is possible that the "stimulatable" lymphocytes may not be actively synthesizing the appropriate IgG molecule or IgG chain at the time of stimulation; it may only be required to have the potential mechanism for synthesis, which may provide, in some unknown way, cell fixed antigenic determinants with which the appropriate antiserum may react to induce transformation.

**SUMMARY**

Lymphocytes from the peripheral blood of newborn rabbits heterozygous for IgG allotypes A$s^4$ and A$s^5$, or A$s^5$ and A$s^6$, obtained at an age when only the maternal allotypic determinants are detectable in the serum, may be stimulated in vitro to transform into "blast" cells with antiallotype sera directed against the determinants controlled both by the maternal and by the paternal chromosomes. This result rules out the possibility that allotypic specificity is conferred upon lymphocytes by environmental IgG and suggests that the lymphocytes of newborn rabbits have the potential to synthesize IgG determinants either in the form of intact IgG molecules or constituent polypeptide chains.

**BIBLIOGRAPHY**


