MATERNAL IMMUNIZATION TO PATERNAL ANTIGENS IN MULTIPAROUS MICE

M. G. BAINES,* J. ANSELL,† A. McLAREN AND H. S. MICKLEM

Departments of Zoology and Animal Genetics, University of Edinburgh

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Summary. Mice of the CBA strain which had given birth to five litters sired by males of a different H-2 type (C57BL/10) showed evidence of immunization to paternal antigens, in that the numbers of lymphocytes forming allocusters with paternal erythrocytes were significantly elevated. On the other hand, the graft-versus-host responsiveness of maternal splenic lymphocytes to paternal antigens remained virtually unchanged, showing only a slight increase at Day 8 of the assay.

INTRODUCTION

The immunological relationship between the mother and the developing fetus is complex and is not as yet fully understood. There is evidence of the development of cellular sensitization of the multiparous female against paternal antigens (Sören, 1967; Maroni & Parrott, 1973; Butsch, Meeker & Grace, 1969), but little or no evidence that such events have any adverse effect on fetal development (Hašková, 1961; Clarke, 1971; Beer & Billingham, 1971). Experimental immunization of female rats with paternal antigens may result in runt disease after birth (Beer, Billingham & Yang, 1972; Beer & Billingham, 1973). The present experiments were designed to look for evidence of immunization by paternal antigens in normal multiparous female mice differing from the male at a strong histocompatibility locus. Two assays were used: the cluster (rosette) assay for lymphocytes specifically recognizing paternal-strain erythrocytes (Micklem, Asfi, Staines & Anderson, 1970), and a modification of the localized graft-versus-host (GVH) assay (Ford, Burr & Simonsen, 1970).

MATERIALS AND METHODS

Two-month-old CBA/H and C57BL/10 (B10) mice were reciprocally mated for four or five successive pregnancies. Two assays for immunization to paternal antigens were performed. Negative controls consisted of parallel assays on age-matched virgin female mice and CBA/H females multiparous by syngeneic males. The negative control data were pooled, as similar results were seen in both groups.

* Present address: Department of Obstetrics and Gynaecology, Queen's University, Kingston, Ontario, Canada.

† Present address: A.R.C. Unit of Reproductive Physiology and Biochemistry, Huntingdon Road Cambridge.

Cluster (rosette) assay

As described by Micklem & Anderson (1972), 5×10^6 spleen lymphocytes freed of erythrocytes by a wash in tris-buffered ammonium chloride (Boyle, 1968) were rolled overnight at 4°C in phosphate-buffered saline, pH 7.2, containing 1% normal serum of the maternal strain, with 15×10^6 red blood cells from either syn-, allo- or xenogeneic sources. The normal maternal serum was previously absorbed for 30 min at 37°C with $\frac{1}{10}$ vol. of both packed paternal erythrocytes and sheep red blood cells.

A modification of the reading procedure was employed as follows. Permanent glass-slide chambers were constructed using a catalysed resin adhesive to attach strips of No. 1 coverslips to the slide to make two chambers/slide (Text-fig. 1).

TEXT-FIG. 1. Diagram of the permanent precalibrated rosette counting chamber. A, standard glass slide, 75×25 mm; B, three strips of No. 1 coverglass; C, No. 3 coverglass, 22×50 mm.

The dimensions were approximately $25 \text{ mm} \times 20 \text{ mm} \times 0.15$ to 0.20 mm, the depth being accurately measured by a micrometer and etched onto the end of the slide. The chamber dividers were lubricated with pure mineral oil and a No. 3 coverslip was placed on top. Before pipetting $50 \mu\text{l}$ of the suspension into a chamber, $2 \mu\text{l}$ fluorescein diacetate solution (5 mg/ml acetone) was added for each ml of cell suspension to allow an estimation of viability (Rotman & Papermaster, 1966). The edges of the chamber were then sealed with mineral oil to prevent evaporation. Total and viable cell counts were then made under tungsten and blue-violet illumination, respectively, utilizing a precalibrated eye-piece grid and the measured depth correction factor. The suspensions were scanned for clusters at $\times 200$ magnification and the presence of a viable yellow-green fluorescent lymphocyte in the cluster was verified. Leucocyte morphology and the number of bound erythrocytes were determined at $\times 400$ to 600 magnification.

Graft-versus-host assay

The reactivity of the thymus-derived (T) cell population was assessed by a

modification of the local assay of Ford *et al.* (1970). Spleen lymphocytes (5×10^6) from a maternal-strain donor were injected into the left rear foot pads of groups of at least four 2-month old F_1 hybrids of the maternal and paternal strains. The hybrid mice were given an intraperitoneal injection of 5×10^{-8} M-5-fluoro-2'-deoxyuridine 4 or 8 days later. After 1 hr, 1 μ Ci of [125 I]5-iodo-2'-deoxyuridine was similarly injected. The left and right popliteal nodes were excised 2 hr later and were fixed in 4% formaldehyde-70% ethanol. After washing in 70% ethanol, the nodes were counted in a Packard Autogamma Scintillation Counter. Uptake was expressed as the ratio of counts in the left (experimental) lymph node to the counts in the right (control) lymph node.

RESULTS

The results of cluster assays on multiparous mice, tested within 20 days after the end of the final pregnancy, and on control mice, are shown in Table 1. The number of allo-(paternal) reactive cluster-forming lymphocytes was elevated in CBA and B10 females that had given birth to five litters sired by the allogeneic paternal strain. The results for the two combinations did not differ significantly from one another, though the increase in allocuster-forming cells only reached the 5% level of significance in the CBA females. The combined increase was significant at the 2% level. Normal CBA mice showed a similar elevation of allocuster-forming cells after a skin allograft (Table 1). The frequencies of syn-(maternal) and xeno- (sheep erythrocyte) cluster-forming lymphocytes were not increased after five pregnancies (Table 2).

There was no significant difference in the GVH responsiveness of multiparous maternal lymphocytes compared to that of lymphocytes from control mice as assayed at Day 4 after inoculation into the F_1 hybrid recipient (Table 3). The Day-8 assay did suggest an increased responsiveness of lymphocytes from multiparous CBA females. Although statistically significant at the 5% level, the increase was counterbalanced by a slightly decreased reactivity in the Day-4 assay.

DISCUSSION

In principle, immunization of the mother to paternal antigens derived from the fetus could involve T-lymphocytes, B-lymphocytes, or both. The GVH assay may be assumed to reflect primarily T-lymphocyte activity. Our data show no clearly significant evidence of increased GVH reactivity in the spleen cells of multiparous mice. By contrast, Maroni & Parrott (1973), using a basically similar GVH assay, showed progressively increasing reactivity in CBA females which had borne one to five litters sired by AKR males. This difference may be explained by two differences in the experimental design. (1) In addition to spleen, Maroni & Parrott (1973) used lymph nodes draining the uterus as a source of lymphocytes; these lymph nodes might be expected to be more strongly sensitized. (2) Our strain combination involved a difference at the H-2 locus, while in the experiments of Maroni & Parrott (1973) both strains were H-2^k, histoincompatibility residing only in the relatively 'weak' antigens

Table 2. Geometric mean frequencies of cluster forming lymphocytes from multiparous and control mice of both mating combinations

Group	Cluster type and frequency/10 ⁶ viable spleen lymphocytes		
	Synclusters (Maternal RBC)	Alloclusters (Paternal RBC)	Xenoclusters (Sheep RBC)
Multiparous	35.5	49.0	218.8
Control	24.3	28.2	222.5
Student's <i>t</i>	<i>t</i> ₃₈ = 1.50	<i>t</i> ₄₄ = 2.45	<i>t</i> ₃₈ = 0.10
Significance	<i>P</i> > 0.10	<i>P</i> < 0.02	<i>P</i> > 0.9

Further details of the mice, and of the allocluster scores, are given in Table 1.

Table 3. Local graft-versus-host response of control and multiparous maternal spleen lymphocytes in 2-month-old hybrid recipient mice

Donor	Day of assay	Ratio of uptake in popliteal lymph nodes after injection of lymphocytes from spleen of:		Student's <i>t</i> and <i>P</i> value
		Control mice	Multiparous mice	
C57BL/10 (H-2 ^b)	4	0.29 ± 0.04 (1.95)	0.38 ± 0.03 (2.39)	<i>t</i> ₁₀₂ = 1.75, <i>P</i> > 0.05
	8	0.30 ± 0.06 (1.99)	Not done	—
CBA/H (H-2 ^k)	4	0.55 ± 0.04 (3.51)	0.46 ± 0.06 (2.87)	<i>t</i> ₅₉ = 1.24, <i>P</i> > 0.20
	8	0.37 ± 0.06 (2.35)	0.55 ± 0.06 (3.51)	<i>t</i> ₂₈ = 2.06, <i>P</i> < 0.05

The hybrids were C57BL/10 × CBA/H F₁ (H-2^{bk}) and the response was measured by uptake of [¹²⁵I]5-iodo-2'-deoxyuridine. Values are given as the logarithmic mean ± one S.E. Figures in parentheses represent the antilogarithm of the mean uptake ratio.

determined by other H-loci. It has long been known that the effect of immunization on GVH reactivity is much greater in weakly histoincompatible strain combinations than in combinations in which a strong H-2 incompatibility exists (Simonsen, 1962; Loutit & Micklem, 1962).

Lymphocytes specifically forming clusters with donor-strain erythrocytes (allocluster-forming cells) have been described in the regional lymph nodes of mice with skin allografts (Micklem *et al.*, 1970; Viklický, Lengerová, Matoušek & Boubelík, 1971). The present results show that such cells are also to be found in the spleens of mice with skin allografts and, in comparable numbers, of multiparous mice. It is not yet certain whether allocluster-forming cells belong to the T-lymphocyte or B-lymphocyte population or to both. Evidence on the sensitivity to anti-theta serum of 'background' syncluster-forming cells in normal mouse lymph nodes (Micklem & Anderson, 1972) and of allocluster-forming cells in mice with skin allografts (M. Boubelík, M. Rose & V. Viklický, personal communication) suggests that many, if not all, are B-cells. The same conclusion has recently been reached by Brondz, Kotomina, Jeliseyeva,

Egorova & Snegiröva (1973). This would be consistent with the presence of the haemagglutinating antibodies to paternal antigens noted by previous authors (Goodlin & Herzenberg, 1964; Kaliss & Dagg, 1964).

The data are compatible with the view, derived from the results of several authors, that maternal sensitization to paternal antigens of the fetus occurs frequently in mice (Sörén, 1967; Maroni & Parrott, 1973), but that its effects do not normally include an accelerated rejection of paternal skin grafts during pregnancy or damage to the developing fetus (Breyere & Barrett, 1960; Prehn, 1960). This failure to express the state of sensitivity is probably due to the coincidental development of enhancing or blocking antibodies (Kaliss & Dagg, 1964; Hellström, Hellström & Brawn, 1969). A possible explanation of the observed increase in the number of allocluster-forming lymphocytes is that they are involved in the production of such blocking factors.

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