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Experimental erythrocyte autoimmunity

I. MICE CONGENIC FOR IMMUNOGLOBULIN ALLOTYPES VARY IN PRODUCTION OF AUTOANTIBODIES BUT PRODUCE SUPPRESSOR CELLS NOT RESTRICTED BY ALLOTYPES

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Summary. Erythrocyte autoantibodies can be elicited in mice by injections of rat RBC which are cross-reactive with mouse RBC. This report shows that induction of autoantibodies is dependent, in part, on gene(s) outside the H-2 complex. Using CBA mice congenic for Ig allotype and the F_1 and F_2 hybrids, a higher incidence of autoantibody production was observed in mice bearing the Ig allotype 1^b ($1^{b/b}$ or $1^{a/b}$) in contrast to mice homozygous for the allotype Ig-1^a. Serum haemagglutination titres against rat RBC were not reduced in the groups of mice with the lower incidence of autoantibody production. A probable explanation for these observations is that the change in Ig allotype is associated with some change in the variable region determining autoimmune specificity that is governed by V_H genes linked to allotype genes. The transfer of 30×10^6 spleen cells from Coombs' positive mice to syngeneic recipients before starting the immunization regime with rat RBC suppressed autoantibody production and enhanced antibody production against rat RBC. These suppressor cells were effective in congenic mice and in F_1 hybrids, which suggest that the Ig allotype is not a crucial site for the effector stage of suppression of this autoimmune response.

Abbreviations: RBC, red blood cells; Ig, immunoglobulin; PBSG, phosphate buffered saline and glucose.

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INTRODUCTION

Mice of many strains produce erythrocyte autoantibodies when injected with rat RBC. The mice also develop in their spleens cells that suppress autoantibody production if transferred to syngeneic recipients before the first injection of rat RBC. These cells do not suppress the response against rat RBC as measured by serum haemagglutination titres (Playfair & Marshall-Clarke, 1973; Cox & Keast, 1973, 1974; Naysmith & Elson, 1977; Cooke, Hutchings & Playfair, 1978; Cox & Finlay-Jones, 1979).

In this paper, two strains of mice selected as congenic for the Ig-1 allotype have been investigated to determine whether the allotypic differences are associated with different levels of autoimmune responsive-

ness and whether suppressor cells for autoantibody production are effective in congenic mice.

MATERIALS AND METHODS

Animals

C_3H, BALB/c and CBA/Ca mice (Ig allotype 1^a) and C57/Bl mice (Ig allotype 1^b) were obtained from the Clinical Research Centre (CRC) and CBA/Ca-b20 mice (Ig allotype 1^b) from the National Institute for Medical Research (U.K.). F_1 hybrids were bred at the CRC by mating female CBA/Ca mice with CBA/Ca-b20 mice and F_2 hybrids by mating randomly the F_1 hybrids. For experiments, mice of both sexes aged 6–10 weeks were used. Mice were kept under conventional conditions. WAG rats were obtained from Carshalton (U.K.) and Sprague Dawley (SD) rats from the CRC.

Immunization protocol

See Cox & Finlay-Jones, 1979.

Coombs' tests

About 40 μl of venous blood was collected into a capillary tube and washed three times in phosphate-buffered saline with glucose (PBSG) of pH 7·4 and an osmolarity of 325 mosM (Cunliffe & Cox, 1979). The Coombs' serum (rabbit anti-mouse IgG) was diluted appropriately (1/400, 1/2000, 1/4000, 1/12,000) in PBSG to which had been added gamma-globulin-free horse serum to a final concentration of 1%. One drop of each dilution of the Coombs' serum or of the buffer and horse serum was mixed with one drop of washed RBC at a concentration of about 1% in Cooke Microtitre Trays (Sterilin, U.K.). After 1 h at 37° and again after overnight at 4°, the suspensions were examined for agglutination. Suspensions of RBC that settled to a small button in the PBSG but did not settle in one or more of Coombs' serum dilutions were recorded as Coombs' positive. For Coombs' tests performed to determine the Ig allotype of the autoantibody, mouse anti-allotype fluid was used (dilutions 1/200, 1/800) in place of rabbit anti-mouse IgG serum.

Haemagglutination of rat RBC

Doubling dilutions of mouse serum in PBS (pH 6·8) supplemented to a final concentration of 1% with gamma-globulin-free horse serum were mixed with an equal volume of a 1% suspension of WAG rat RBC in PBS in Cooke Microtitre Trays (Sterilin, U.K.). The trays were incubated for 1 h at 37° and then overnight at 4° and examined for agglutination. The results are expressed as the geometic mean of the titres.

Adoptive transfer of spleen cells

The procedure has been described (Cox & Finlay-Jones, 1979). Briefly, spleen cell donors remained untreated or were injected each week with 0·2 ml of a 6% suspension of WAG rat RBC and 34 days after the first injection, spleens from mice in each group were pooled, single cell suspensions prepared, and 30×10^6 viable nucleated cells injected intravenously into each mouse. About 18 h after spleen cell transfer, the recipients were given the first injection of rat RBC.

Detection of Ig allotypes

Mouse anti-Ig-1^a serum was kindly provided by Dr Frank Shand (Wellcome, U.K.). Mouse anti-Ig-1^b was prepared by W.R.T. Briefly, BALB/c mice were immunized with serum from C57/Bl mice that had been grafted with BALB/c skin and injected with BALB/c lymphocytes. After several booster injections the mice were given an ascites tumour and fluid collected 5–7 days later. This protocol favours the production of antibodies reactive with Ig-l(IgG2a) allotypes, but weak reactions to allotypes on other classes and subclasses are not precluded (Herzenberg & Herzenberg, 1978). Sera from mice to be tested were reacted with the anti-allotype fluid on microscope slides coated with 1% Oxoid special agar dissolved in PBSG. After 18 h at about 15°, the slides were examined for precipitin lines. Sera from F_2 mice reacting with both anti-allotype fluids were classified as heterozygous (Ig-$1^{a/b}$) and serum from mice reacting with one anti-allotype fluid but not the other, as homozygous (Ig-$1^{a/b}$ or Ig-$1^{b/b}$). The specificity of the anti-allotype fluids was confirmed using sera from CBA/Ca-b20 and C57/Bl mice (Ig-1^b) and BALB/c, C_3H and CBA/Ca mice (Ig-1^a) in immunodiffusion assays.

Statistics

The analysis was made using Fischer's exact test for 2×2 contingency tables.

RESULTS

Two strains of CBA mice, selected as congenic for the Ig-1 allotype, showed a highly significant difference in susceptibility to induction of erythrocyte autoantibodies caused by injections of rat RBC (Table 1).

Table 1. Differences in susceptibility to induction of erythrocyte autoantibodies in CBA mice selected as congenic for immunoglobulin allotypes

Mouse strain	Rat RBC injected‡		Days after first injection of rat RBC 13	20	28
CBA/Ca	SD	1·5%	0/5†	0/5	1/5
CBA/Ca-b20	SD	1·5%	4/12	12/12***§	12/12**
CBA/Ca	SD	6%	0/10	2/10	4/9
CBA/Ca-b20	SD	6%	6/12*	12/12***	11/11**
CBA/Ca	WAG	1·5%	0/5	1/5	2/5
CBA/Ca-b20	WAG	1·5%	4/10	10/10**	10/10*
CBA/Ca	WAG	6%	1/11	2/11	4/10
CBA/Ca-b20	WAG	6%	7/12*	12/12***	12/12**

† Number of mice Direct Coombs' test positive/total number of mice in group.

‡ Mice were injected intraperitoneally with 0·2 ml of rat RBC on days 0, 7, 14 and 21.

§ The asterisks show significant differences between the two strains of mice using Fishers's exact tests. * $P<0·05$; ** $P<0·01$; *** $P<0·001$.

Autoantibodies, detected using direct Coomb's tests, were readily induced in CBA/Ca-b20 mice (Ig-1^b) in contrast to CBA/Ca mice (Ig-1^a). This difference occurred when RBC from two different strains of rats were used as immunogens and at two doses of rat RBC (Table 1). Autoantibodies in the F_1 hybrid of these two strains of mice were readily induced by injections of rat RBC, which suggests that the propensity to make autoantibodies is determined by a dominant gene (or genes).

Since the two congenic strains of mice were bred and reared at different places, it is possible that the difference in susceptibility to autoantibody production was caused by different environmental influences affecting the immune system during its ontogeny. This possibility was investigated by breeding F_1 mice (CBA/Ca-b20 × CBA/Ca) and then mating the F_1 mice randomly to obtain F_2 mice. In the F_2 mice it was possible to test the association of Ig allotype with susceptibility for autoantibody production in mice that had been bred and reared under the same conditions. The Ig allotypes of the F_2 mice were established using mouse anti-Ig allotype fluids and mouse serum in immunodiffusion assays. The incidence of autoantibody production in

Table 2. Susceptibility to induction of erythrocyte autoantibodies segregated with immunoglobulin allotypes in F_2 mice [F_1 × F_1 (CBA/Ca × CBA/Ca-b20)]

Ig allotype of mice		$1^{a/a}$			$1^{b/b}$			$1^{a/b}$		
Days after first injection* of rat RBC		13	20	26	13	20	26	13	20	26
Incidence of Positive Coombs' tests with anti-	IgG	0/21	4/21	11/21	11/17	15/17	17/17	18/35	28/35	35/35
	Ig-1^a	0	0	2/21	0	0	0	0	2/35	2/35
	Ig-1^b	0	0	0	0	12/17	13/17	11/35	16/35	23/35
Serum† titres to rat RBC		—	—	910 (270)	—	—	665 (180)	—	—	590 (210)

* Mice injected i.p. on days 0, 7, 14 and 21 with 0·2 ml of a 6% suspension of RBC from SD rats.

† Geometric mean titre (standard deviation).

Table 3. Autoantibody production suppressed by spleen cells from congenic mice of different immunoglobulin allotypes

Spleen cell donors* mouse strain	WAG rat RBC† injected into spleen cell donors	Recipient strain	Days after first injection of rat RBC: Direct Coombs tests 14	21	27	34	Serum titres to rat RBC 34
CBA/Ca	Yes	CBA/Ca	0/9‡	0/9	0/9	0/9	12,800
CBA/Ca	No	CBA/Ca	1/9	1/9	2/9	2/9	7,600
CBA/Ca	Yes	CBA/Ca-b20	0/6	0/6	0/6	0/6	11,000
CBA/Ca	No	CBA/Ca-b20	3/7	7/7	7/7	7/7	2,900
CBA/Ca	Yes	F_1	0/7	0/7	0/7	0/7	14,000
CBA/Ca	No	F_1	1/7	1/7	6/7	6/7	2,540
CBA/Ca-b20	Yes	CBA/Ca-b20	0/9	0/9	0/9	0/9	14,600
None	—	CBA/Ca-b20	3/10	8/10	10/10	10/10	2,100
CBA/Ca-b20	Yes	CBA/Ca	0/8	0/8	0/8	0/8	10,800
CBA/Ca-b20	Yes	F_1	0/8	0/8	0/8	0/8	13,400
F_1§	Yes	F_1	0/7	0/7	0/7	0/7	14,900
None	—	F_1	3/8	5/8	6/8	8/8	3,200

* Spleens taken 34 days after first weekly injections of WAG rat RBC, or from untreated mice.
† Mice injected i.p. at weekly intervals with 0·2 ml of a 6% suspension of RBC from WAG rats.
‡ Number of mice DCT positive/total number of mice.
§ F_1 (CBA/Ca × CBA/Ca-b20).

the F_2 mice, following injections of rat RBC, was significantly lower in the progeny homozygous for Ig-1^a in contrast to mice that were either homozygous for Ig-1^b ($P<0{\cdot}007$) or heterozygous (Ig-$1^{a/b}$; $P<0{\cdot}00002$) (Table 2). Coombs' tests performed using anti-allotype fluids in place of anti-IgG sera showed that in the heterozygous F_2 mice, a higher incidence of autoantibodies were detected using anti-Ig-1^b in contrast to anti-Ig-1^a (Table 2). The anti-Ig-1^a fluid detected a high incidence of erythrocyte autoantibodies in C_3H mice (Ig-1^a) which were positive in Coombs' tests with anti-mouse IgG sera after 8 weekly injections of rat RBC. (Cox & Cox, unpublished data). This shows that the anti-Ig-1^a fluid could be used to detect autoantibodies of that allotype. The antibody response to rat RBC did not correlate with the incidence of autoimmunity in any group and was observed to be highest in the group with the lowest incidence of autoantibodies.

Previously it had been shown that spleen cells from mice positive in direct Coombs' tests after several injections of rat RBC suppress autoantibody production in syngeneic recipients if transferred before the first injection of rat RBC. The results listed in Table 3 demonstrate that spleen cells from mice of either Ig allotype (1^a or 1^b) suppress autoantibody production in syngeneic mice, congenic mice and in the F_1 hybrid. In contrast, the response to rat RBC, as measured by serum haemagglutination titres, was not suppressed by the spleen cells and in all cases was higher in mice receiving spleen cells from Coomb's positive mice before the rat RBC injections, which suggests that primed spleen cells were being transferred.

DISCUSSION

The proportion of mice becoming Coombs' positive when injected with rat RBC has been shown to vary in different strains (Cox & Keast, 1973, 1974; Cooke & Playfair, 1977). In this report it is demonstrated that for one inbred strain (CBA), variation in susceptibility to induction of erythrocyte autoantibodies occurs independently of the H-2 haplotype. A likely explanation for the differences between the two strains of mice selected as congenic for Ig allotypes is that a V_H gene (or genes) linked to the genes for Ig allotype determines whether the mice can produce autoantibodies with specificity for their erythrocytes. In other systems it has been shown that antibody production to some antigens is determined by V_H genes linked to the genes for Ig heavy chain allotypes (Dorf, Dunham, Johnson & Benacerraf, 1974; Riblet, Blomberg, Weigert, Lie-

berman, Taylor & Potter, 1975; Mäkelä, Kaartinen, Pelkonen & Karjalainen, 1978; Fernandez, Lieberman & Möller, 1979).

However, other explanations cannot be excluded including the one, as the autoimmune response is T-cell dependent (Playfair & Marshall-Clarke, 1973), that genes linked to the Ig allotype determine whether T cells co-operate with the potentially autoimmune B cells. It is unlikely that the decreased responsiveness is due to active suppressor mechanisms because in the F_1 hybrid responsiveness is dominant. Further, transfer of unprimed spleen cells from the low responder strain (CBA/Ca) to the high responder strain (CBA/Ca-b20) or the F_1 hybrid before the first injection of rat RBC did not suppress autoantibody production (Table 3). This result argues against suppressor cells causing the lower responsiveness, especially as primed spleen cells from one congenic strain were shown to be effective suppressors in the other congenic strain.

The presence of the Ig allotype 1^b is not always associated with a high incidence of erythrocyte autoimmunity following injections of rat RBC. Previously it had been observed that C57/Bl mice (Ig-1^b) are poor responders in this model system (Cox & Keast, 1973; 1974; Cooke & Playfair, 1977) In contrast to the results presented here, CBA mice (Ig-1^a) have been used as high responders (Cooke & Playfair, 1977). Similarly C_3H mice (Ig-1^a) have been used as high responders (Cox & Keast, 1974). Thus the propensity to make RBC autoantibodies after injections of rat RBC appears to be dependent on factors in addition to the genes associated with Ig allotypes. The observation that in heterozygous F_2 mice (Table 2) and in F_1 mice (unpublished data), the autoantibodies of allotype 1^b are more common than those of allotype 1^a is compatible with the idea of allelic exclusion (Cebra, Colberg & Dray, 1966). Thus responding autoimmune B cells would secrete antibodies of one allotype but not both. For the strains of mice investigated the B cells most likely to respond would be those with receptors associated with the allotype 1^b.

The suppression of the autoimmune component of the anti-rat RBC response by cells from mice of one Ig allotype in congenic mice of the other allotype and in the F_1 hybrid suggests that Ig allotypes are not crucial targets for the effector stage of suppression. Because the transfer of primed spleen cells causes an increase in the response to the various rat RBC antigens as measured by serum haemagglutination tests, (Table 3, Naysmith & Elson, 1977; Cooke *et al.*, 1978) it can be concluded that the suppressor cells are epitope specific in their activity and are not simply releasing antibodies which block the immunogeneicity of rat RBC. In another model system, suppressor cells were shown to inhibit Ig production of a certain allotype by removing allotype-specific T helper cells required to activate B cells of that allotype (Herzenberg, Okumura, Cantor, Sato, Shen & Boyse, 1976). In this model of autoimmunity possible targets for the suppressor cells appear to be the idiotypes of the autoimmune B cells and/or of the relevant T helper cells (e.g. see Hetzelberger & Eichmann, 1978).

However, on the basis of the present results arguments can be made against the idea that B-cell idiotypes are the targets for suppression. If the difference in susceptibility to autoantibody production in the two congenic strains is due to V_H region differences of the receptors of autoimmune B cells, it follows that the idiotypes are likely to be different. Accordingly, idiotype-specific suppressor cells directed against B cells from one congenic strain would not be effective in the other congenic strain. The results (Table 3) show that the suppressor cells were effective in either congenic strain. Thus it seems possible that either the suppressor cells are not directed against the idiotypes of the autoimmune B cells or, that the difference in susceptibility to autoantibody production is not due to V_H region differences of autoimmune B cells in the two congenic strains. These possibilities require further investigation.

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