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Lack of autoantigen-specific splenic suppressor cells in mice with an X-linked B-lymphocyte defect

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Summary. Male and female progeny of a cross between CBA/N female and BALB/c male mice were tested for their ability to generate autoantigen-specific suppressor cells as a result of stimulation with cross-reacting rat RBC. Male mice, hemizygous for the X-linked defect, were unable to generate these antigen-specific suppressor cells, whereas their female littermates behaved like the normal (CBA/Ca × BALB/c)F_1 mice.

INTRODUCTION

It has been shown by several groups that immunization of normal mice with rat RBC results in the production of antibody with activity directed towards mouse RBC (Playfair & Marshall-Clarke, 1973; Cox & Keast, 1974; Naysmith & Elson, 1977), and furthermore that suppressor cells specific for the autoimmune response exist in the spleens of these animals (Naysmith & Elson, 1977; Cooke, Hutchings & Playfair, 1978; Gare & Cox, 1978).

CBA/N mice display an X-linked genetic defect which manifests itself in a number of B lymphocyte functional abnormalities. These mice are unable to respond to those T-independent antigens which stimulate B cells appearing late in ontogeny, and their responses to some T-dependent antigens may also be impaired. When (CBA/N × BALB/c)F_1 hybrids were immunized with rat RBC, it was found that the male progeny of this cross were deficient in their ability to produce anti-mouse RBC autoantibody, whereas the female progeny were able to produce antibody in amounts equivalent to that of a normal CBA/Ca or (CBA/Ca × BALB/c)F_1 mice (Marshall-Clarke, Cooke & Hutchings, 1979). In contrast, both sexes produced comparable amounts of anti-rat antibodies. In this report we present evidence that defective (CBA/N × BALB/c)F_1 male mice failed to generate antigen-specific suppressor cells in this system, although suppressor activity was normal in their female littermates.

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MATERIALS AND METHODS

Mice

(CBA/N × BALB/c)F_1, (CBA/Ca × BALB/c)F_1 and BALB/c mice were bred in our own animal house using female CBA/N and female CBA/Ca mice obtained from the NIMR, Mill Hill, London. The CBA/N mice were a generous gift from Dr G. G. B. Klaus. The mice used for the primary rat RBC immunization were all 8–12 weeks old.

Rat RBC

Rat RBC were obtained by cardiac puncture of Wistar

rats maintained in our own colony. The rat RBC were washed three times before injecting 2×10^8 RBC i.p. into mice to generate an (anti)autoantibody response as described previously (Playfair & Marshall-Clarke, 1973).

Generation of antigen-specific suppressor cells

Antigen-specific suppressor cells were generated as described previously (Cooke *et al.*, 1978). Briefly, mice received four weekly injections of 2×10^8 rat RBC i.p. and 8–10 weeks later they were boosted with 2×10^8 rat RBC i.p. One week after boosting, their spleens were removed, and 50×10^6 thrice washed splenocytes were transferred i.v. to histocompatible recipients as a source of suppressor cells.

Assay of autoantibody activity

Direct antiglobulin (Coomb's) test: DCT. Mice were bled from the retro-orbital venous plexus and the red cells washed four times in isotonic saline before being tested. A single batch of rabbit anti-mouse immunoglobulin (shown by immunoelectrophoresis to react against IgG and IgM) was stored in small aliquots at $-20°$ and used at a standard final dilution of 1:80. Agglutination was scored microscopically on a scale ranging from positive, visible only under the microscope (1), to a massive agglutination involving all the cells (4), after 30 min incubation with antiserum at room temperature. Positive and negative controls were included in all determinations. This more rapid qualitative assay shows a complete correlation with a more quantitative assay based on titration of the developing antiserum.

Statistical analysis

Analysis of the differences between the DCT of different groups of mice was carried out using the Wilcoxon ranking test (Wilcoxon, 1978).

In vitro *generation of suppressor cells with Con A*

Pooled mouse spleen cells were washed three times and cultured in petri dishes at 10^7/ml in RPMI 1640, 5% FCS and 5 mM glutamine containing 4 μg/ml Con A (ICN Pharmaceuticals Ltd). All media and supplements were obtained from Flow Labs, Irvine, Scotland. Cultures were carried out in a humidified incubator at 37° in an atmosphere of 5% CO_2 in air. After 48 h the cells were harvested, washed three times, and then cultured for a further 5 days to estimate their suppressor activity in an *in vitro* antibody response.

In vitro *antibody production*

Mouse spleen cells were cultured in a modified Marbrook-Diener culture system (Feldman & Basten, 1971) using 15×10^6 fresh primed spleen cells to which 2×10^6 suppressor cells and 2×10^7 SRBC were added. The culture medium used was RPMI 1640 supplemented with 5% FCS, 5 mM glutamine, 100 μg/ml streptomycin and 100 units/ml penicillin G. The cultures were harvested after 5 days and the number of antibody-forming cells was estimated using the modification of the haemolytic plaque assay described by Cunningham and Szenberg (1968). Cultures were set up in triplicate, and the results are expressed as arithmetic means ± standard deviation.

RESULTS

Antigen-specific suppressor cells in CBA/N mice

Defective male (CBA/N × BALB/c)F_1 and normal female (CBA/N × BALB/c)F_1 mice immunized with rat RBC produce equivalent amounts of agglutinating antibody to rat RBC although when autoantibody production is measured by DCT, the response of the defective males is markedly reduced compared with their female littermates (Marshall-Clarke *et al.*, 1979). Spleen cells from rat RBC primed female or male (CBA/N × BALB/c)F_1 mice were transferred into female or male (CBA/Ca × BALB/c)F_1 mice, and their ability to suppress the subsequent induction of autoantibody by rat RBC in the recipients analysed. Figure 1 shows a representative result of three experiments, statistical analysis of the data shows that no significant suppression is seen by spleen cells of the male progeny ($n=14$) whereas the suppression of DCT at week 4 mediated by the female progeny is highly significant ($2\alpha=0{\cdot}01$, $n=15$).

It can also be seen from Fig. 1 that both female and male (CBA/Ca × BALB/c)F_1 mice respond equally well to the cross-reacting antigens in terms of the autoantibody induced. Therefore it is clear that the defective males are not only inadequate in mounting an autoantibody response, but also deficient in their ability to generate those suppressor cells which specifically control the autoantibody component of the response to rat RBC.

Non-specific suppressor cells in CBA/N mice

To test the possibility that the lack of these suppressor cells in the (CBA/N × BALB/c)F_1 males was due to a

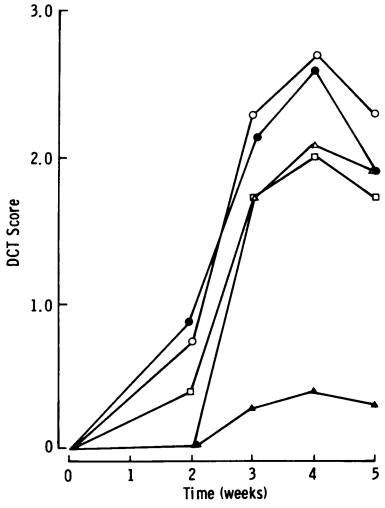

Figure 1. Autoantigen-specific suppressor activity of male and female (CBA/N × BALB/c)F_1 mice. ●, ♀(CBA/Ca × BALB/c)F_1 rat RBC only; ○, ♂(CBA/Ca × BALB/c)F_1 rat RBC only; ▵, ♂(CBA/N × BALB/c)F_1 suppressor cells into ♀(CBA/Ca × BALB/c)F_1; □, ♂(CBA/N × BALB/c)F_1 suppressor cells into ♂(CBA/Ca × BALB/c)F_1; ▲, ♀(CBA/N × BALB/c)F_1 suppressor cells into ♀(CBA/Ca × BALB/c)F_1. 50×10^6 suppressor cells were injected i.v. 24 h before the first i.p. injection of rat RBC. All mice received 2×10^8 rat RBC i.p. at weekly intervals for 4 weeks.

generalized defect in suppressor T-cell activity, the ability of Con A to generate non-specific suppressor cells in spleen cell suspensions from male and female (CBA/N × BALB/c)F_1 mice was examined. It can be seen from Table 1 that both defective and normal spleen cells can be stimulated to an equivalent extent by Con A to generate cells capable of non-specifically suppressing an *in vitro* secondary immune response. This suppression is not due to simple passive carry-over of Con A in the spleen cells, since spleen cells cultured for 24 h with Con A are not capable of suppressing either a primary or a secondary immune response *in vitro*. (Cooke, Heppel, Hutchings & Roitt, 1979). It would therefore seem that the lack of antigen-specific suppressor cells in the defective male is not due to a more generalized defect of suppressor function.

DISCUSSION

In recent years extensive investigation of the X-linked defect of the CBA/N mouse has been carried out (Scher, Steinberg, Berning & Paul, 1975; Cohen, Scher & Mosier, 1976). It has previously been shown that the male progeny of the (CBA/N × BALB/c)F_1 mouse, hemizygous for the X-linked defect characteristics of this strain, are unable to mount the normal autoantibody response elicited by rat RBC, although their heteroantibody response to this antigen is not impaired (Marshall-Clarke *et al.*, 1979). We have argued that this defect of autoantibody production might be another example of arrested B-cell maturation, or might result from more easily tolerized B cells in the defective males. An alternative explanation could be that deficient autoantibody production is the result of overzealous suppressor cells in (CBA/N × BALB/c)F_1 males. This latter possibility was tested in this report. Our results demonstrate that, on the contrary, there is a lack of significant suppression in male progeny compared with the females. The lack of autoantibody

Table 1. Con-A induced suppressor activity

Responder cells	Suppressor cells	SRBC	PFC culture	
			Direct	Indirect
Exp. 1 (CBA/Ca × BALB/c)F_1 ♀	None	+	1540 ± 580*	2020 ± 780
	(CBA/Ca × BALB/c)F_1 ♂	+	700 ± 180	540 ± 160
	(CBA/N × BALB/c)F_1 ♀	+	540 ± 440	580 ± 400
	(CBA/N × BALB/c)F_1 ♂	+	300 ± 60	320 ± 160
	None	−	140 ± 60	240 ± 120
Exp. 2 (CBA/Ca × BALB/c)F_1 ♂	None	+	2480 ± 680	2460 ± 560
	(CBA/N × BALB/c)F_1 ♂	+	80 ± 20	120 ± 50
	(CBA/N × BALB/c)F_1 ♀	+	100 ± 40	126 ± 42
	(CBA/Ca × BALB/c)F_1 ♀	+	120 ± 72	220 ± 86
	None	−	20 ± 34	60 ± 34

* Values are arithmetic means of three cultures ± SD.

production in these defective mice is not therefore due to excessive suppressor cell activity.

We have shown (Cooke, Hutchings & Nayak, 1980) that CBA/nu/nu mice do not make rat agglutinins or autoantibody, nor do they generate suppressor activity in response to immunization with rat RBC. All these activities are therefore T dependent. We and others (D. Naysmith, personal communication) also find that there is an anti-Thy 1·2 *insensitive* component to the specific suppressor activity of normal rat RBC primed spleen cells. This would suggest that the antigen-specific suppression seen in this system may be due to the concerted activity of suppressor T cells, idiotype specific T helper cells and B cells specific for the idiotype of the autoantibody. Leaving aside the possibility of an as yet undisclosed T-cell abnormality in mice with the CBA/N defect, it would seem that the lack of suppressor cells specific for the autoimmune response could be explained either by a lack of a B-cell class which is an inducer for T suppressor cells(Ts), or by a lack of the relevant idiotype(s) and a consequent inability to generate idiotype-specific T suppressor cells. If the latter were the case, we would propose that the autoantibody production itself, or the expansion of a clone of B cells bearing the idiotype of the autoantibody, is the driving force for the generation of suppressor cells. The defective (CBA/N × BALB/c)F_1 male, by not producing the autoantibody, would therefore fail to generate suppressor activity. These proposals are currently under investigation.

It is interesting to note that in a recent report Bottomly, Mathieson, Cosenza & Mosier (1979) have shown that mice with the CBA/N defect which have low circulating levels of T15 idiotype, also fail to generate idiotype-specific suppressor T cells.

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REFERENCES

BOTTOMLY K., MATHIESON B.J., COSENZA H. & MOSIER D.E. (1979) In: *B Lymphocytes in the Immune response* (Ed. by M. Cooper, D.E. Mosier, I. Scher and E.S. Vitetta, p. 323. Elsevier/North Holland, Amsterdam.

COHEN P.L., SCHER I. & MOSIER D.E. (1976) *In vitro* studies of the genetically determined unresponsiveness to thymus independent antigens in CBA/N mice. *J. Immunol.* **116,** 301.

COOKE A., HUTCHINGS P.R. & PLAYFAIR J.H.L. (1978) Suppressor T cells in experimental autoimmune haemolytic anaemia. *Nature (Lond.)*, **273,** 154.

COOKE A., HEPPEL L., HUTCHINGS P. & ROITT I.M. (1979) Con A induced suppressor activity of lymphocytes distinguished by the presence or absence of the Fc receptor cell. *Immunology*, **47,** 90.

COOKE A., HUTCHINGS P.R. & NAYAK R. (1980) Specific and non-specific suppressor cell activity in NZB mice. *Immunology*, **40,** 335.

COX K.O. & KEAST D. (1974) Autoimmune haemolytic anaemia induced in mice immunised with rat erythrocytes. *Clin. exp. Immunol.* **17,** 319.

CUNNINGHAM A.J. & SZENBERG A. (1968) Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology*, **14,** 599.

FELDMAN M. & BASTEN A. (1971) The relationship between antigenic structure and the requirement for thymus derived cells in the immune response. *J. exp. Med.* **134,** 103.

GARE N.F. & COX K.O. (1978) Erythrocyte autoantibody production diminished by autoantibody induced suppressor cells. *Proc. Aust. Soc. med. Res.* **11,** 40.

MARSHALL-CLARKE S., COOKE A. & HUTCHINGS P.R. (1979) Deficient production of anti red cell autoantibodies by mice with an X-linked B lymphocyte defect. *Europ. J. Immunol.* **9,** 820.

NAYSMITH J.D. & ELSON C.J. (1977) Autosuppression of an autoantibody response to erythrocytes in mice. *Abstr. Allergologia et Immunopathologie*, **5,** 480.

PLAYFAIR J.H.L. & MARSHALL-CLARKE S. (1973) Induction of Red Cell Autoantibodies in Normal mice. *Nature (New Biol.)*, **243,** 213.

SCHER I., STEINBERG A.D., BERNING A.K. & PAUL W.E. (1975) X-linked B lymphocyte immune defect in CBA/N mice. II. Studies of the mechanisms underlying the immune defect. *J. exp. Med.* **142,** 637.

WILCOXON F. (1968) In: *Introduction to Probability and Statistics* (Ed.) by H.L. Adler and E.B. Roessler), p. 156. W.H. Freeman and Company, San Francisco.