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Restricted heterogeneity of antibody synthesized by T-cell deprived mice

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Summary. Mice which had been thymectomized and injected with anti-thymocyte serum to remove long-lived recirculating T cells, initially failed to produce haemagglutinating and haemolysing antibody after injection of sheep erythrocytes. After six fortnightly injections of heterologous erythrocytes, however, haemolysin titres in the T-cell deprived mice were comparable to those in similarly challenged but immunologically intact animals. Isoelectric focusing of these sera indicated that the anti-sheep erythrocyte antibody eventually synthesized by the T-cell deprived mice was less heterogeneous than antibodies found in the sera of control mice.

INTRODUCTION

During the course of studies to test the extent of the immune unresponsiveness which could be induced in mice by a combination of thymectomy and administration of rabbit anti-mouse thymocyte serum (ATS), it was found that successive injections of sheep erythrocytes eventually elicited relatively high titres of both haemagglutinating and haemolytic antibody. The much delayed and gradual build up of antibody titres in thymectomized, ATS-treated mice may have been a consequence of these animals having insufficient T cells to stimulate all available B-cell precursors. This, it was thought, might lead to a degree of homogeneity in the spectrotypes of antibodies synthesized by these animals. Consequently the isoelectric spectra (spectrotypes) of the anti-erythrocyte antibodies of deprived and control animals were examined at intervals during a prolonged course of immunizing injections.

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MATERIALS AND METHODS

CBA/Ca mice (formerly CBA/Lac) were thymectomized at 6 weeks of age (Law, Bradley & Rose, 1963). Within 10 days of the operation the experimental group received at 2 day intervals, four injections of 0·25 ml rabbit anti-mouse thymocyte serum (Levey & Medawar, 1966). The mice were rested for 4 weeks before the commencement of erythrocyte injections. Control animals were either thymectomized, or injected with ATS, or received no treatment at all.

Sheep erythrocytes (SRBC) for immunization were obtained preserved in Alsever's solution (Tissue Culture Services Ltd, Slough, Berks.) or were obtained from sheep S-38 at NIMR for use in the IEF-overlay assay (q.v.). They were washed three times in saline before injection. Each mouse received 0·2 ml of a 20% v/v suspension of SRBC intraperitoneally every fortnight (approximately 5×10^8 SRBC/injection). Just prior to the second and subsequent injections each

mouse was bled and the serum separated with the use of 'Sera Sieve' (Hughes and Hughes Ltd). Sera were heat-inactivated (56° for 30 min) and stored at −20°.

Twenty microlitres of each serum was serially diluted (two-fold) in isotonic saline in microtitration plates (Linbro Scientific Co., Conn. U.S.A.) using an electronic pipetting device (Compupet$_{100}$, General Diagnostics, New Jersey, U.S.A.). Haemolysins were assayed by addition of 20 μl 2% v/v SRBC and 20 μl of 10% fresh guinea-pig serum. Haemagglutinins were determined in duplicate titrations in which guinea-pig serum was omitted, but where 20 μl 0·1 M 2-mercaptoethanol was added (Scott & Gershon, 1970).

The isoelectric focusing (IEF) overlay assay was used to visualize antibody spectrotypes in individual sera (Phillips & Dresser, 1973; Dresser, 1978). Allo anti-mouse immunoglobulin sera specific for mouse IgG2a were prepared and tested for specificity (see Dresser, 1978) and used to 'develop' the mouse anti-SRBC antibody (Phillips & Dresser, 1973).

RESULTS

Figure 1 shows the titration results of an experiment in which thymectomized ATS-treated mice were compared with untreated mice and with mice which had been thymectomized alone or given ATS alone.

The thymectomized, ATS-treated group had relatively low titres of haemagglutinating antibodies compared with normal mice throughout the series of ten SRBC injections. In contrast, haemolytic antibody titres in these animals rose to a level comparable to that of normal mice after the sixth injection of antigen. Mice which had been either thymectomized alone or injected with ATS alone had antibody titres indistinguishable from normal mice throughout the time course.

The spectrotypes of specific anti-SRBC antibodies were visualized in the sera from the mice examined in the experiment depicted in Fig. 1. These sera were individually assayed by means of the IEF overlay assay after the first, fourth, seventh and tenth injections of SRBC. Figure 2a and b represent the anti-SRBC IgG2a spectrotypes obtained from normal mice and Fig. 2c and d from mice thymectomized but not injected with ATS. From the fourth injection, IgG2a anti-SRBC antibody in these two types of mice possessed spectrotypes with mean pI distributed throughout a range of pH from 5 to 8; due to their high titre it was necessary to dilute these sera 1 : 10 before applying the 3 μl samples to the IEF plate. Figure 2e and f show the IEF patterns of undiluted sera of two mice from the group given ATS alone.

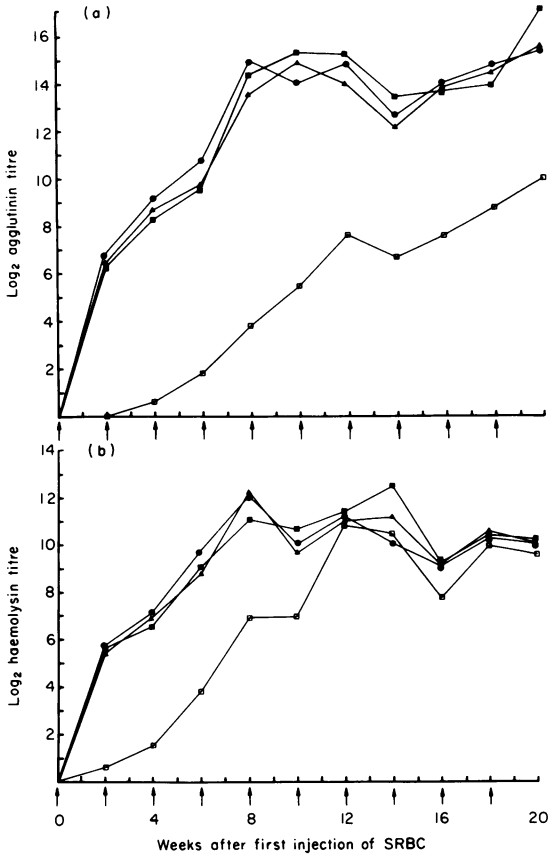

Figure 1. The log$_2$ 2-mercaptoethanol resistant agglutinating (a) and total haemolysing (b) antibody titres in normal mice (●) and mice which had been thymectomized only (▲), injected with ATS only (■) or both thymectomized and injected with ATS (□). Sera were examined 14 days after each of ten fortnightly injections (arrows) of approximately 5×10^8 SRBC.

Undiluted sera from the thymectomized ATS-treated mice were usually found to have IgG2a antibody activity after the seventh or tenth injection of SRBC (Fig. 2g–l) and furthermore, of the four groups of sera tested, the thymectomized ATS-treated mice were found to have the least heterogeneity in the isoelectric points of their IgG2a antibodies. (Eight out of nineteen mice had no anti-SRBC antibody in this IgG subclass detected by IEF, even though all of these apparently IEF-negative mice had minimum log$_2$ hae-

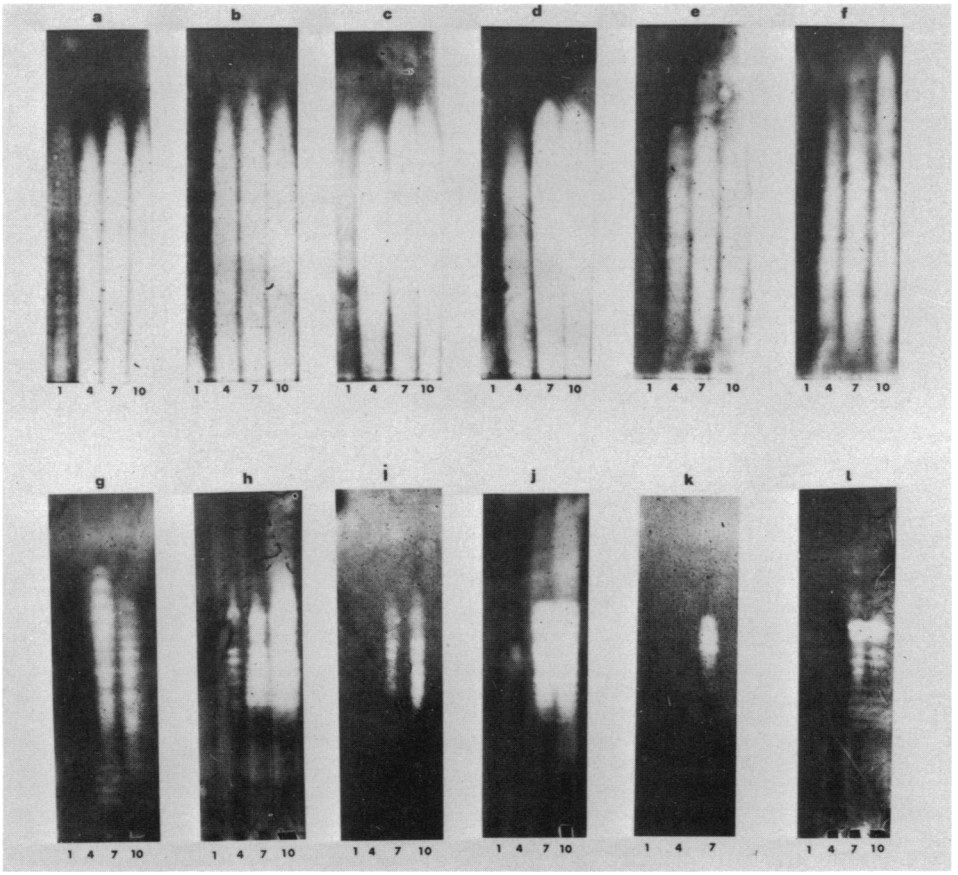

Figure 2. The isoelectric spectra of anti-SRBC antibodies in two untreated mice (a and b) and in two mice which had been thymectomized only (c and d), ATS-injected only (e and f), and in six thymectomized and ATS-injected (g–l). Sera taken from each mouse 14 days after one, four, seven and ten injections of 5×10^8 SRBC were examined on the same IEF plate. Sera a–d were diluted 1:10 before use and sera e–l were used undiluted.

molysin titres of 8 after the seventh injection of SRBC. None of these IEF-negative animals is illustrated in Fig. 2.)

Table 1 gives the 2-mercaptoethanol resistant haemagglutinin and haemolysin antibody titres of the individual mice depicted in Fig. 2. As in Fig. 1, it may again be noted that in the thymectomized ATS-treated mice the $\log_2$ haemolysin titre was typically greater than the agglutinin titre, this being the reverse of the general pattern found in the normal, thymectomized alone or ATS-injected alone groups.

The results depicted in Fig. 2 were obtained with a dilution of mouse serum and a period of incubation with complement source adjusted in a manner which allowed ideal spectrotype analysis of sera from experimental groups having a wide range of antibody titres (Fig. 1). In order to confirm that the apparent restriction of heterogeneity in antibody isoelectric points was not a function of different total antibody titres in different mice, sera with 2-mercaptoethanol resistant agglutinin titres of $\log_2 7$ were selected from each of the four mouse groups for isoelectric focusing under identical conditions (that is, at the same dilution and on the same plate). The result is shown in Fig. 3. In this figure, sera a and b which were taken from normal mice after the first injection of SRBC, demonstrated a wide range of spectrotypes. A similar range is seen in samples c and d which were obtained from thymectomized mice after the first SRBC injection. Samples e and f were from mice which had been both thymectomized and

Table 1. The $\log_2$ 2-mercaptoethanol resistant agglutinin and total haemagglutinin titres in the sera of mice which were examined in IEF in Fig. 2. The antibody titres were estimated 14 days after one, four, seven and ten intraperitoneal injections of approximately 5×10^8 SRBC. Letters a–l correspond to the IEF patterns in Fig. 2

		No. of SRBC injections							
		1		4		7		10	
		Agg	Lys	Agg	Lys	Agg	Lys	Agg	Lys
Normal	a	7	6	15	12	12	11	14	10
	b	7	6	14	12	12	10	16	10
Thymectomy	c	6	4	15	12	12	11	17	10
alone	d	7	6	14	12	11	11	15	11
ATS alone	e	6	5	14	10	14	12	19	10
	f	7	6	TF	12	13	12	18	10
Thymectomy	g	0	0	4	6	8	12	11	9
and ATS	h	0*	5	9	9	9	12	11	9
	i	0	0	0	5	5	10	7	9
	j	0	0	4	8	8	9	13	10
	k	0	0	TF	3	1	7	†	
	l	0	0	4	9	7	10	11	9

* A $\log_2$ total agglutinin titre of 5 was detected in this mouse at this time, but all agglutinating capacity of the serum was destroyed by 2-mercaptoethanol.

† This animal died after the eighth time of bleeding.

TF = Technical failure.

ATS-injected, and the sera were collected after the tenth (sample e) or seventh (sample f) injection of SRBC, at which times each of these two sera had agglutinin titres of 7 as had sera a–d. Restriction in the range of isoelectric points of IgG2a antibodies in mice e and f is readily apparent when they are compared with sera a–d. A similar restriction was found in sera g and h from mice injected with ATS, although in this group the serum samples were obtained from both mice after the first injection of SRBC.

DISCUSSION

Anti-thymocyte serum administered *in vivo* leads to the disappearance of recirculating lymphocytes, most of which are thymus-derived (Leuchars, Wallis & Davies, 1968; Lance, 1970). When ATS is given to thymectomized mice the blood-borne PHA-responsive (T cell) population is decimated, and does not recover over a 400 day time course (Doenhoff & Leuchars, 1977). At least one population of B cells, that is responsive *in vitro* to stimulation with bacterial lipopolysaccharide, appears to be unaffected by thymectomy and ATS treatment (Doenhoff & Leuchars, 1977), although other B cells are somewhat depleted (Anderson, Dresser, Iverson, Lance, Wortis & Zebra, 1972).

Although thymectomy and ATS results in a diminished T-cell pool size, mice so treated are not completely lacking in T cells (Doenhoff & Leuchars, 1977). The delay in the antibody response to injections of sheep erythrocytes could therefore be interpreted on the basis of an initially relatively small population of helper T cells with reactivity for this antigen being expanded through mitosis induced by each successive injection of antigen (Davies, Leuchars, Wallis & Koller, 1966). The reason for haemolysin titres in deprived mice reaching the level found in normal mice while agglutinin titres remain lower than normal, is a matter of conjecture. A likely explanation is that haemolysing antibodies are largely IgM and an IgM response is much less thymus-dependent than an IgG response (Miller & Mitchell, 1968; Taylor & Wortis,

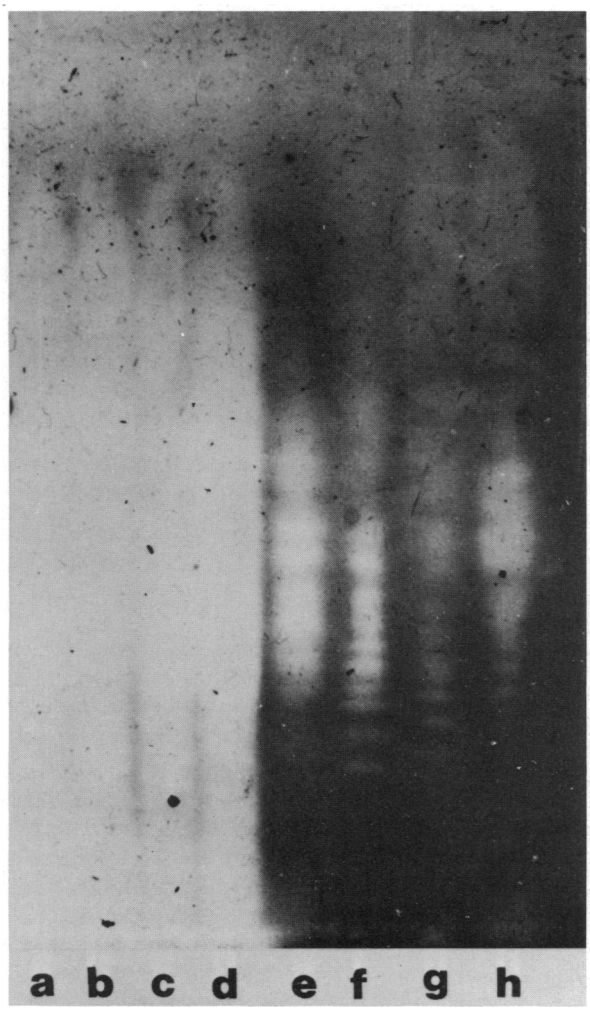

Figure 3. The isoelectric spectra of anti-SRBC antibodies in untreated mice (a and b), and those which had been thymectomized only (c and d), both thymectomized and injected with ATS (e and f) or given ATS only (g and h). All eight sera had 2-mercaptoethanol resistant agglutinin titres of 7. Sera a, b, c, d, g and h were obtained 14 days after one injection of SRBC. Serum e was obtained after ten injections of SRBC and serum f after seven injections.

1968). Both IgM and IgG antibodies probably make significant contributions to the operational class of haemagglutinating antibodies.

Phillips & Dresser (1973) found that normal mice challenged with small numbers of heterologous erythrocytes synthesized antibody of restricted heterogeneity and suggested that this could be a result of only some of the antigenic determinants on the red cells being present in high enough concentration to induce an immune reponse. In the present experiments, 5×10^8 SRBC were administered each time and antigen concentration is therefore unlikely to be the limiting factor. A restriction in the electrophoretic heterogeneity of immunoglobulin molecules has been observed in mice (Van Muiswinkel, Radl & Van der Wal, 1976) and rhesus monkeys (Radl, Van den Berg, Voormolen, Hendricks & Schaefer, 1974) following irradiation and reconstitution with bone marrow. When irradiated reconstituted monkeys were challenged with antigen, the antibodies produced earlier

in the reconstitution time course were more homogeneous than those synthesized later. Restriction of antibody heterogeneity was similarly observed here in non-thymectomized mice given ATS after the first challenge with SRBC (Fig. 3, mice g and h), with a full range of antibody species being found following subsequent antigen challenge. Thus restoration of the capacity to synthesize a full range of antibody species seems to occur during the time that the T-cell pool is recovering in non-thymectomized mice which have been irradiated or treated with ATS (Rozing & Benner, 1976; Doenhoff & Leuchars, 1977), a process which in both situations requires a relatively long period to complete.

No recovery of the T-cell pool occurs in irradiated or ATS-treated mice which have in addition been thymectomized (Doenhoff & Leuchars, 1977). In contrast, B-cell populations in such animals have probably not been deleteriously affected by ATS, and furthermore, the B-cell pool is reconstituted much more rapidly following irradiation and bone marrow therapy (Nossal & Pike, 1973; Rozing & Benner, 1976; Doenhoff & Leuchars, 1977). It therefore appears that restriction in the heterogeneity of antibodies may be a function of the size of the T-cell pool. Since thymus-derived cells are not responsible for the synthesis of circulating antibody (Davies, 1969), it remains to be determined to what degree diminished T-cell populations can, in the presence of intact B-cell populations, have an influence on the range of antibody species which are synthesized following antigenic challenge.

A possible mechanism is suggested by the *in vitro* experiments of Phillips & Waldmann (1977) who demonstrated by using limiting numbers of T helper cells in microcultures that there was a markedly decreased heterogeneity of response to SRBC, compared with microcultures with excess T-help. They concluded that an indivdual T-helper cell co-operates with only one precursor B cell.

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