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The Functions of Immune T and B Rosette-forming Cells

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Summary. From 7 to 35 days after CBA mice were primed with SRBC their spleens were removed and anti-SRBC rosettes were formed. The rosettes were purified from other spleen cells by velocity sedimentation at 4° and rosette-enriched, rosette-depleted and various control populations were injected into lethally irradiated CBA recipients. These were challenged with SRBC and their spleens analysed for direct (IgM) and enhanced (IgG) PFC 7 days later. Removal of RFC depleted the primed spleen cells of their capacity adoptively to transfer an immune response. This depletion was antigen-specific. Purified rosettes alone prepared 7–8 days after priming transferred significant (relative to controls) immune reactivity to the irradiated recipients. Both B and T RFC were present at this stage and the response was dependent upon cell collaboration between these two populations. Later in the primary response (35 days) purified rosettes transferred negligible immune reactivity. But these RFC (90 per cent B) collaborated with rosette-depleted cells to restore full reactivity. B memory lymphocytes (AFCP) form rosettes from 7 to 35 days after immunization but T memory cells only do so for a limited stage during the peak of the primary response. The majority of T memory cells probably never form rosettes in this system. It is suggested that most T RFC may be cells mediating delayed hypersensitivity or are 'passive' rosettes.

INTRODUCTION

A number of different lymphoid populations will form rosettes depending upon the type of red cell or particulate antigen used, the immune status and species of the lymphocyte donor and the particular experimental methods used to prepare the rosettes. Rosette-formation may be non-specific—many human T lymphocytes will form rosettes with sheep red blood cells (SRBC) (Coombs, Gurner, Wilson, Holm and Lindgren, 1970; Lay, Mendes, Bianco and Nussenzweig, 1971), while most B lymphocytes will bind red cells coated with antibody and complement (Michlmayr and Huber, 1970; Jondal, Holm and Wigzell, 1972). However, under carefully defined experimental conditions rosette-forming cells (RFC) are antigen-binding cells (ABC) reacting specifically with an antigen (Biozzi, Stiffel and Mouton, 1967; Zaalberg, 1964; Bankhurst and Wilson, 1971). Removal of RFC to a specific antigen from an immunologically competent population depletes that population of reactivity to the antigen used (Bach, Mullner and Dardenne, 1970).

It has been shown that background RFC include most antibody-forming cell precursors (AFCP) to their antigen (Brody, 1970; Osoba, 1970; Gorczinski, Miller and Phillips, 1971). But the roles of RFC from immunized animals have not been fully elucidated. Some antibody-forming cells (AFC) form rosettes (McConnell, 1971) but with the rosette

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technique used in this study most AFC are excluded (Wilson, 1971a). Both B and T lymphocytes form rosettes in immunized mice (Schlesinger, 1970; Greaves and Moller, 1970; Wilson and Miller, 1971), though there is some discrepancy between workers in the relative proportions of the two types. The function of B and T RFC from immunized animals has been investigated in the present study. It is shown that most AFCP (B cells) from immunized mice will form rosettes but most T memory cells will not. It is suggested that T RFC may be cells mediating delayed hypersensitivity.

MATERIALS AND METHODS

Mice and immunization

Male CBA/Har/Wehi mice aged 10–12 weeks at the time of immunization were used. Sheep red blood cells (SRBC) and donkey red blood cells (DRBC) were collected into Alsever's solution, stored at 4° and washed three times in normal saline before use. Mice received i.p. injections of 10^9 RBC as indicated by the protocol below.

Antisera used

Anti-θ C3H serum was raised in AKR mice by the method of Reif and Allen (1964). Rabbit anti-mouse IgG was kindly provided by Dr M. Feldmann and used in *in vitro* assays for antibody-forming cells (AFC).

Irradiation

Recipient CBA mice were given total body irradiation in a Philips (RT 250) X-ray machine. The dose was 800 rads to mid point with maximum back scatter at 15 mA and a half value layer of 0·8 mm^3.

Rosette preparation

Mice were killed by cervical dislocation, their spleens were removed and teased through a fine stainless steel mesh. The cells were washed twice in Eagles' minimum essential medium (Grand Island Biological Co.) buffered to pH 7·3 with 'hepes' (*N*-2-hydroxy-ethyl-piperazine-*N*-2-ethanesulphonic acid (CalBiochem.) (HEM). Rosettes were then prepared as previously described (Wilson, 1971a) as modified from McConnell, Munro, Gurner and Coombs (1969). Briefly, 6-ml suspensions of 10^7 spleen cells/ml were mixed with 5×10^7 SRBC/ml in phosphate buffered saline, pH 7·3, with 10 per cent foetal calf serum (FCS) (Commonwealth Serum Laboratories Parkville) in 10·0-ml plastic conical centrifuge tubes. The suspension was syringed through 26-gauge needles to disrupt cell clumps and then centrifuged at 400 ***g*** for 7 minutes at 4°. After a further 10 minutes the pellets were gently resuspended with a Pasteur pipette and rosettes were counted in Neubauer white cell counting chambers. Only cells completely surrounded or almost surrounded by RBC were classed as rosettes; these usually had 8–12 RBC attached.

Experimental protocol

The general experimental design is summarized in Fig. 1. Spleen cells were harvested from CBA mice 7–35 days after they had received a single i.p. injection of 10^9 SRBC and/or DRBC. Rosettes were prepared as above and were then separated from other cells in the suspension by velocity sedimentation. The cell suspension in HEM was layered onto a 13·0-ml 50–100 per cent continuous FCS gradient in 25×6-cm round-bottomed glass

tubes. FCS fractions were appropriately diluted with HEM before being layered to form the gradient. Rosettes were allowed to settle for approx. 100–115 minutes (the time being recalibrated for fresh FCS batches) and the gradient was then carefully removed in three layers and the number of rosettes and white cells counted in each. The upper two 3·0-ml layers were depleted of rosettes and the bottom 3·0-ml layer was enriched. Details of contaminating rosettes and free white cells in respective layers are given in the results. The top layer contained only occasional rosettes but on occasions the middle layer had to be

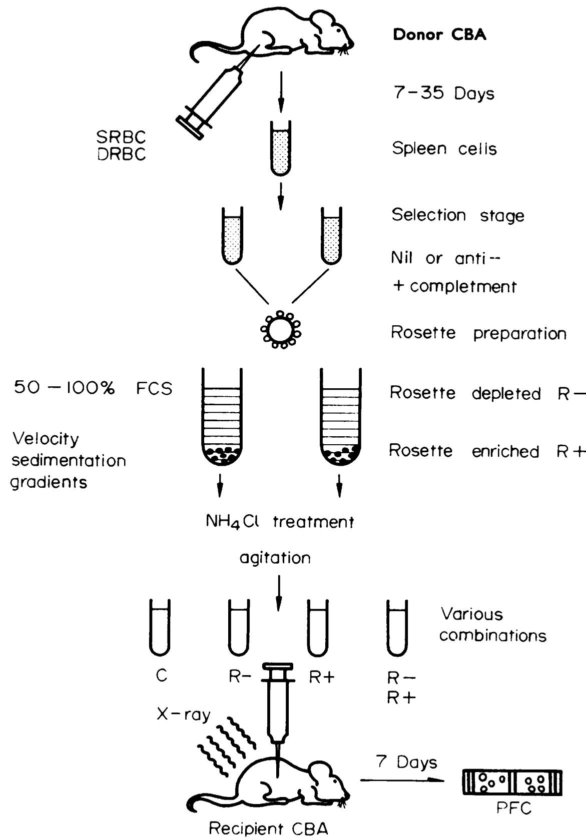

FIG. 1. Experimental protocol.

centrifuged to spin down contaminating rosettes to provide a fully rosette-depleted preparation. The full procedure from rosette formation through to the end of velocity sedimentation was undertaken at 4°.

Recipient irradiated mice in groups of five or six received one or other of the following cell populations: (a) 3×10^6–10^7 spleen cells from primed mice as control. These cells had been centrifuged and sedimented in parallel with the other cell populations but had not had rosettes formed. (b) 3×10^6–10^7 spleen cells from the same donors as (a) depleted of their rosettes by velocity sedimentation. (c) Rosettes purified by velocity sedimentation. Their numbers were equivalent to the total RFC calculated to be injected with control population (a). (d) A mixture of (b) and (c) as a reconstituted population. (e) Control

cells from primed mice as in (a) but in low numbers $0{\cdot}3–1 \times 10^6$ cells equivalent to the numbers of contaminating white cells in the rosette enriched sample (c). This was to exclude the activity of the rosette-enriched populations being largely due to contaminating spleen cells not forming rosettes.

In one series of experiments, to assess the effect of depletion of T RFC, spleen cells from primed mice were treated with either AKR anti-θ C3H serum or normal AKR serum for 30 minutes at 37°. After three HEM washes the cells were incubated for a further 30 minutes at 37° with a 1:6 dilution of guinea-pig serum as a source of complement. After a further two washes in HEM, rosettes were prepared as before and the cell samples were separated and injected. It was argued that rosettes with adherent RBC might readily be trapped in lung capillaries. To reduce this possibility rosette-enriched, and all other cell populations to be injected, were washed once in HEM and the cell pellets resuspended in Tris-buffered 0·9 per cent NH_4Cl to lyse adherent RBC on rosettes. The suspensions were warmed to 37° over 10 minutes in a water bath, then centrifuged and washed in HEM before being made up to their final concentration for i.v. injection in Eagles' minimum essential medium. Samples were injected in 0·5 ml and recipient mice then received 10^9 SRBC (or DRBC) i.p. After 7 days they were killed, their spleens removed, teased, washed, and assayed for AFC.

Assay for antibody-forming cells

AFC were assayed by the haemolytic plaque assay method of Cunningham and Szenberg (1968). IgG secreting AFC were enumerated by the addition of 0·01 ml of rabbit anti-mouse IgG (1:60 dilution) to the assay chambers.

RESULTS

The results of a typical standard experiment are presented in Table 1. Spleen cells from mice immunized with SRBC 13 days before, when depleted of their RFC to SRBC, had defective anti-SRBC function on transfer into irradiated hosts. Enhanced AFC (IgG-secreting) were more affected than direct (IgM) AFC. Purified rosettes alone transferred some anti-SRBC activity, and significantly more than could be attributed to the contaminating cells present in the rosette fraction (390 compared with 90 direct AFC/spleen and 2770 compared with 460 enhanced AFC respectively). Thus the rosette preparation appeared to carry representative cells of the various collaborative populations necessary for generating a humoral response. However, the purified rosette fraction transferred only 20 per cent of the immune reactivity of the control fraction suggesting that a very large component of specifically reactive cells were not forming rosettes.

Recombining the two fractions—rosette-enriched and rosette-depleted cells—totally restored the immune response relative to controls on transfer. The response was nearly three-fold the sum of enhanced AFC and twice the sum of direct AFC from transfer of the individual populations suggesting that some cell collaboration had occurred between the rosettes and the rosette-depleted cells.

It can be seen from Table 1 that the degree of rosette purification after velocity sedimentation was about twelve-fold. In general this ranged from ten to twenty-fold throughout the experiments. As the contribution of contaminating cells to the immune response of the irradiated hosts was so trivial (1–2 per cent of control) there was no need to improve the purification beyond that obtained.

TABLE 1

PFC IN SPLEENS OF IRRADIATED CBA MICE RECEIVING ROSETTE-ENRICHED AND ROSETTE-DEPLETED POPULATIONS FROM SPLEENS OF MICE PRIMED 13 DAYS BEFORE

Cells injected	PFC/recipient spleen ± SE	
	Direct	Enhanced
10^7 spleen cells (33,000 RFC)	2050 ± 802	34,950 ± 10,608
10^7 rosette-depleted spleen cells	950 ± 235	6680 ± 2767
33,000 rosettes (+800,000 contaminating cells)	390 ± 64	7220 ± 2569
10^6 spleen cells	90 ± 29	460 ± 197
10^7 rosette-depleted spleen cells + 33,000 rosettes	2850 ± 495	37,800 ± 7937

Donor mice primed with 10^9 SRBC.

SPECIFICITY OF ROSETTES IN TRANSFERRING IMMUNOLOGICAL REACTIVITY

Mice were primed with both SRBC and DRBC. After 35 days their spleens were removed, mixed SRBC plus DRBC rosettes were made and the spleen cells were depleted of both rosette types. In other preparations pure SRBC or DRBC rosettes were made and the irradiated recipients received: (a) control primed spleen cells; (b) spleen cells depleted of both DRBC and SRBC rosettes; (c) fraction (b) plus purified SRBC rosettes; (d) fraction (b) plus purified DRBC rosettes. Details of AFC in recipient spleens analysed 7 days later are given in Table 2.

As before, removal of both types of rosettes depleted the spleen cell population of immunological reactivity to both antigens, DRBC and SRBC. Reconstitution of the depleted population before transfer with rosettes of one type restored the AFC response in recipients to that antigen but not to the other. Therefore the rosette fractions contained a major component of specific immune capacity.

THE EFFECT OF TIME AFTER PRIMING ON THE IMMUNE CAPACITY OF ROSETTE-FORMING CELLS

(a) *Depletion*

Table 3 summarizes the results of twelve experiments where spleen cells, taken at different times after SRBC-priming, were depleted of their RFC by velocity sedimentation before being injected into irradiated hosts. The AFC response in the recipients' spleens 7 days later showed that depletion of IgG-secreting capacity became increasingly efficient as the duration from priming extended (68 per cent depression at 7 days and 87 per cent at 35 days $P = <0{\cdot}05$). In contrast no significant alteration was seen in direct (IgM) AFC numbers (66 per cent depletion at 7 days and 72 per cent at 35 days).

Table 2

Specificity of immune response of transferred rosettes*

Cells injected	PFC/recipient spleen ± SE			
	SRBC		DRBC	
	Direct	Enhanced	Direct	Enhanced
3×10^6 spleen cells (7500 S–RFC 6000 D–RFC)	1610 ± 417	23,937 ± 5280	275 ± 34	2880 ± 614
3×10^6 spleen cells rosette-depleted (S+D)	250 ± 35	5050 ± 2302	70 ± 12	130 ± 34
3×10^6 spleen cells rosette-depleted +7500 S– rosettes	2530 ± 391	35,900 ± 4992	80 ± 29	490 ± 148
3×10^6 spleen cells rosette-depleted +6000 D–rosettes	420 ± 125	6950 ± 1320	280 ± 75	2480 ± 665

* Donors primed with 10^9 SRBC and 10^9 DRBC 35 days before.
S–RFC indicates RFC with SRBC. D–RFC indicates RFC with DRBC.

Table 3

The effect of depletion of rosettes on the immunological memory of primed spleen cells

Duration of priming	Number of experiments	PFC/recipient spleen (per cent of controls)	
		Direct	Enhanced
7	4	34	32
10	1	24	24
13	2	34	15
35	5	28	13

Irradiated CBA mice received 10^7 primed spleen cells depleted of rosettes. Control mice received 10^7 spleen cells. PFC measured after 7 days.

Table 4

Enrichment of PFC response of irradiated CBA mice given purified rosettes from primed mice

Duration of priming (days)	Number of experiments	7-day PFC/recipient spleen (per cent of control)	
		Direct	Enhanced
7	4	1060	1430
13	2	300	800
35	4	310	290

Control mice received unseparated spleen cells equivalent in numbers to rosettes + contaminating cells.

(b) *Enrichment*

Purified rosettes on transfer to irradiated recipients could generate an AFC response to SRBC which was considerably higher than that from primed spleen cell controls, equivalent in number to the sum of rosettes plus contaminating cells (Table 1). Table 4 summarizes the extent of this enriched response. Rosettes taken 7 days after immunization exhibited on transfer, ten to fourteen-fold enrichment over an equivalent number of control cells. However, with increasing time after primary immunization the degree of enrichment declines reaching only about three-fold after 35 days.

TABLE 5

PFC RESPONSE OF IRRADIATED CBA MICE RECEIVING ROSETTES PURIFIED FROM 10^7 SRBC-PRIMED SPLEEN CELLS COMPARED WITH MICE RECEIVING 10^7 PRIMED-SPLEEN CELLS

Duration of priming	Number of experiments	7-day PFC/recipient spleen (per cent of control)*	
		Direct	Enhanced
7	5	36	28
13	2	15	11
35	3	6	2

* Control mice received 10^7 spleen cells containing x RFC from SRBC immunized mice.
The other mice received x RFC, purified as rosettes.

This declining effect is more pronounced when the AFC response in mice receiving the purified rosette population is compared with that of mice receiving control primed cells containing an equivalent number of RFC (Table 5). While the rosettes can mount a response almost one-third of the control cells when they come from 7-day immunized mice this falls to 2–6 per cent 35 days after priming.

Thus, although the efficiency of depleting spleen cells of specific IgG immune reactivity by removal of rosettes increases with time after priming the cell donor, the rosettes themselves show decreased reactivity without recombination with other cell fractions.

THE T CELL CONTRIBUTION TO ROSETTE-ENRICHED AND ROSETTE-DEPLETED POPULATIONS

Spleen cells from mice primed 8 days before were treated with AKR anti-θ serum and complement to lyse θ-positive cells before preparation of rosettes. Other cells from the same donors were treated with AKR serum and complement. Then various cell fraction combinations were injected into irradiated recipients. Results of one experiment are detailed in Table 6.

Depletion of AKR serum-treated rosettes, as before, reduces the immune capacity of the depleted spleen cells to SRBC on transfer. The purified rosette population contained 40 per cent θ-positive RFC and, in the recipients, produced 308 direct and 2525 indirect AFC in response to SRBC compared with 20 and 95 AFC respectively when equal numbers of control cells (rosettes+contaminants) were injected. After anti-θ treatment the purified rosettes had totally lost their capacity to transfer any significant immune response, so this capacity must be dependent upon the presence of θ-positive (T) RFC. When this θ-negative rosette population was recombined with the rosette-depleted fraction full immune reactivity to SRBC was restored suggesting that sufficient θ-positive,

TABLE 6

PFC IN SPLEENS OF IRRADIATED CBA MICE RECEIVING ANTI-θ-TREATED OR NON-TREATED ROSETTES AND SPLEEN CELL POPULATIONS FROM 8-DAY-PRIMED MICE

Sample injected	PFC/recipient spleen+SE	
	Direct	Enhanced
5×10^6 spleen cells (23,500 RFC)	1310±309	12,950±2114
5×10^6 rosette-depleted spleen cells	710±68	1770±694
23,500 rosettes (400,000 contaminating cells)	308±86	2525±559
14,000 θ-negative rosettes (400,000 contaminating cells)	6±6	31±19
4×10^5 spleen cells	20±15	95±28
14,000 θ-negative rosettes + 5×10^6 rosette-depleted spleen cells	1262±133	11,231±2019
23,500 rosettes + 5×10^6 θ-negative rosette-depleted cells	545±149	3550±1201

Spleen cell donors primed with 10^9 SRBC i.p. 8 days before.

TABLE 7

PFC IN SPLEENS OF IRRADIATED CBA MICE RECEIVING CELL POPULATIONS FROM 5 DAY OR 37-DAY-PRIMED MICE OR MIXTURES FROM THE TWO GROUPS

Cells injected	PFC/recipient spleen±SE	
	Direct	Enhanced
5-day rosette-depleted cells (5×10^6)	190±48	840±201
5-day rosettes (25,000+4·5 $\times 10^6$ contaminants)	660±145	3920±660
5-day rosettes+5-day rosette-depleted cells (25,000+5×10^6)	870±186	5300±1792
37-day rosettes (9,000+ 4·0 $\times 10^5$ contaminants)	2110±182	7460±785
37-day rosette-depleted cells (5×10^6)	260±77	3620±1497
37-day rosettes+37-day rosette-depleted cells (9,000+5×10^6)	6350±729	94300±11268
5-day rosettes+37-day rosette-depleted cells (25,000+5×10^6)	1450±133 (expected 2300)	13437±143 (expected 8300)
37-day rosettes+5-day rosette-depleted cells (9,000+5×10^6)	2770±441 (expected 920)	34100±6083 (expected 7540)

Donor mice primed with 10^9 SRBC.

non-rosette-forming cells must have been present in the rosette-depleted fraction. When untreated rosettes were mixed with anti-θ-treated rosette-depleted cells there was only a slight restoration in cell numbers above that of the pure rosettes alone. Thus the θ-positive RFC included only a small proportion of the θ-positive cells necessary for restoration of full immune reactivity.

THE EFFECT OF COMBINING VARIOUS FRACTIONS FROM MICE IMMUNIZED 5 DAYS AND 37 DAYS BEFORE

The results of these experiments are summarized in Table 7. Recombination of rosette-enriched and rosette-depleted fractions from 5-day primed mice reveals almost no evidence of cell collaboration between the 2 fractions, almost all the activity being already present in the RFC population. But, after 37 days the effects of recombination are dramatic. The sum of direct AFC from rosettes and rosette-depleted cells is 2370/spleen which rises to 6350 for the combined population. But the IgG AFC increase from 11080/spleen for the individual fractions to 94,300 for the recombined populations, a nine-fold rise.

When rosettes from 5- or 37-day primed mice are mixed with rosette-depleted cells from the other group of donors evidence of significant cell collaboration occurs only when 37-day rosettes are combined with 5-day rosette-depleted cells. Here, after transfer, the sum of direct AFC from the two fractions separately is one-third that of the combination and for enhanced AFC is one-fifth. As 37-day rosette-depleted cells give evidence of good collaborative potential with 37-day rosettes (see above) then 5-day rosettes must lack some feature allowing a significant collaborative response with the 37-day rosette-depleted cells.

DISCUSSION

The experiments described above demonstrate that, after immunization, most B memory cells (AFCP) form rosettes while most T memory cells do not, even when up to 40 per cent of the rosettes are θ-positive. It is also shown that cell collaboration occurs between RFC and rosette-depleted cells in a secondary response and, for a limited time, between T and B RFC.

Removal of RFC to SRBC and DRBC from spleen cells of mice primed with both antigens depletes these cells of their capacity to transfer an adoptive immune response to irradiated animals (Table 2). Responsiveness to one antigen is restored by replacing the RFC to that antigen alone. Thus RFC are necessary for a secondary response and carry antigenic specificity. Evidence is also presented for cell collaboration between RFC and rosette-depleted cells. This occurs at all stages of the immune response but is most clearly seen by 35 days after priming when the response in the irradiated host of the two populations together is up to nine-fold the sum of the individual populations (Table 7). At this stage <10 per cent of the RFC are θ-positive suggesting that B RFC are collaborating with T memory cells which are not forming rosettes. Table 6 shows that ablation of T RFC by treatment of the cells with anti-θ and complement does not impair their ability to collaborate with the rosette-depleted cells supporting the concept that AFCP are included within the RFC population. The same Table shows that removal of T cells, by anti-θ treatment, from the rosette-depleted cells blocks the collaboration between them and the

RFC. That is, AFCP (B cells) are very largely within the RFC population while T memory (helper) cells are in the rosette-depleted group.

During the peak of the immune response 40–50 per cent of RFC prepared by the present method are θ-positive (Table 6, Wilson and Miller, 1971). At this stage purified RFC alone can adoptively transfer some immune reactivity though this capacity declines from 7 days after immunization and by 35 days is negligible (Tables 4 and 5). However, at 7 days purified rosettes transfer a response more than ten-fold that of an equivalent number of control cells. Following anti-θ treatment the purified rosettes transfer a negligible secondary response implying that collaboration can occur between the θ-positive and θ-negative RFC populations, that is between T and B RFC (Table 6). Does the θ-positive population contain all, or most of the T memory cells at this stage? The combination of anti-θ-treated RFC and rosette-depleted cells which exhibits full immune reactivity on transfer indicates that the B RFC are finding sufficient non-rosette-forming T helper cells for full activity. The converse experiment (anti-θ-treated rosette-depleted cells plus RFC) confirms that the θ-positive RFC cells do not include most T memory cells as there is little further restoration of activity above that of the purified rosettes alone.

Bach *et al.* (1970) have clearly shown that removal of RFC from spleen cells of unprimed mice results in a loss of immunological reactivity to the antigen used. Osoba (1970) Brody (1970) and Gorczinski *et al.* (1971) deduced that background RFC acted functionally as AFCP (B cells). The present study establishes identical conclusions for primed RFC: that they act functionally as AFCP. Co-operation between B and T cells in a secondary response has been described by Miller and Sprent (1971) for chicken γ-globulin, and by Cunningham and Sercarz (1971) for SRBC and both groups have deduced that immunological memory is a property of both B and T cells. The experiments described above confirm that cell co-operation is necessary for a humoral response to xenogeneic red cells in the secondary response and provides some evidence that both T and B cell memory is necessary. Table 7 shows that 5-day primed RFC interact with 37-day primed rosette-depleted cells to give $13{,}437 \pm 143$ enhanced PFC in recipient mice whereas when both populations have been primed for 37 days the recipient response is $94{,}300 \pm 11{,}268$. Cunningham and Sercarz (1971) suggest that T cell memory develops rapidly over 2–4 days after immunization while B cell memory increase slowly up to 8 weeks. As most B cells reactive with SRBC are in the RFC population the 5 day RFC with poor B cell memory might be responding poorly to the good T cell memory of the 37 day rosette-depleted cells containing most T cells.

The nature of the T cell antigen-binding receptor is controversial (Vitetta, Bianco, Nussenzweig and Uhr, 1972). T cells carry little or no surface Ig compared with B cells (Raff, Sternberg and Taylor, 1970; Nossal, Warner, Lewis and Sprent, 1972; Hammerling and Rajewsky, 1971). Detection of T-ABC depends very largely upon the rosette technique as autoradiographic methods with radio-labelled antigens detect only B cells (Bankhurst and Wilson, 1971). Hogg and Greaves (1972) have shown that T RFC are inhibited by pretreatment of the cells with anti-L chain, anti-Fab and some but not all anti-μ antisera. However, there are great variations in the proportion of T and B RFC to SRBC detected by different workers. Bach and Dardenne (1972) and Greaves and Moller (1970) find up to 70 and 50 per cent respectively of background RFC are θ-positive when SRBC are used as antigen. Following immunization 35–50 per cent of RFC are θ-positive (Greaves and Raff, 1971). In contrast Schlesinger (1970) and Wilson and Miller (1971)

find θ-positive rosettes to SRBC occur only during the peak of an immune response. In view of the observation of Lay, Mendes, Bianco and Nussenzweig (1971) that many human and some T lymphocytes of other species will form non-specific rosettes with SRBC, depending upon the temperature conditions selected for the procedure, the finding of θ-positive rosettes to SRBC must be very carefully reassessed. There is little doubt in the present study that T RFC to SRBC will collaborate with B RFC and are therefore largely specific anti-SRBC RFC. However, most T memory cells have not formed rosettes and, late in the response (35 days), there are very few θ-positive RFC. What then is the role of T RFC? As most T memory cells do not form RFC it is possible that these represent cells mediating delayed hypersensitivity, perhaps with some helper activity. But recently Crone, Koch and Simonsen (1972) have suggested that T RFC may arise from IgM antibody produced during an immune response binding onto soluble antigen gently held by T cell receptors, then forming a lattice to which SRBC attach by free-binding sites. This is an attractive hypothesis which could explain the limited appearance of T RFC following immunization, when they carry some collaborative capacity. It would also explain why T RFC in thymus-educated mice (Wilson and Feldmann, 1972, unpublished) are so low, and it could provide some explanation for the super-addition phenomenon where, during an immune response the sum of inhibition of rosette formation of anti-α, γ and μ becomes >100 per cent (Greaves, 1971, Wilson, 1971b).

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