

## SELECTIVE PRIMING OF T CELLS BY CHEMICALLY ALTERED CELL ANTIGENS\*

BY GUNTHER DENNERT† AND DEREK F. TUCKER§

(From The Salk Institute for Biological Studies, The Armand Hammer Center for Cancer Biology, San Diego, California 92112)

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At least three different types of cells are needed for a humoral response to most antigens. The bone marrow-derived lymphocyte (B cell) produces the humoral antibody (1, 2) and is triggered by a cooperating thymus-derived lymphocyte (T cell) to do so (3). A phagocytic cell, the macrophage ( $M\phi$ ), is often needed to "process" or "handle" the antigen (4, 5) for this cooperation. The details of these cell interactions are not complete and several models have been proposed (6-9) for them. We have tried to interfere with some aspects of antigen processing by  $M\phi$  to see whether such processed antigen is necessary for T cell priming, B cell priming, or for both and for the cooperating events between B and T cells. To do this, we treated erythrocytes with formaldehyde or glutaraldehyde and tested their immunogenicity in vivo and in vitro.

Chicken erythrocytes (CRBC) so treated show no gross changes in antigenicity judged by their agglutination patterns with antibody prepared against native CRBC. Table I shows that CRBC fixed with formaldehyde (F-CRBC) have the same agglutinability with anti-CRBC antibody as untreated cells, while somewhat higher titers were usually found with glutaraldehyde-fixed CRBC (G-CRBC), probably because they tend to clump spontaneously. But untreated and fixed red cells probably are processed or handled differently by  $M\phi$ .<sup>1</sup> Recently it has been shown that  $M\phi$  readily phagocytose formalinized sheep erythrocytes (SRBC) (10), although they do not phagocytose untreated SRBC or CRBC (11), unless anti-red cell antibody is present. When  $M\phi$  from animals immunized with red cells are incubated with the same red cells overnight, they release in a soluble form a material (4) which is immunogenic in Mishell-Dutton type cultures (12).

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† Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

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<sup>1</sup> Macrophages referred to in this paper are peritoneal exudate cells (PEC) which are obtained 2-4 days after intraperitoneal injection of peptone broth (4).

Thus supernatants obtained after incubation of a suspension of CRBC, together with immune PEC, could be shown to effectively stimulate an anti-CRBC response by cultured spleen cells (Table II). Supernatants from a

TABLE I  
*Agglutination of CRBC, F-CRBC, and G-CRBC by Anti-CRBC Antiserum*

Agglutination titer on		
CRBC	F-CRBC	G-CRBC
2 <sup>9</sup>	2 <sup>9</sup>	2 <sup>10</sup> -2 <sup>11</sup>

Anti-CRBC serum was raised by intraperitoneal injection of 0.2 ml 10% CRBC suspension into B6D2F<sub>1</sub> (C57BL/6J × DBA/2J) mice for 3 wk. F-CRBC were prepared by adding an 8% suspension of red cells in phosphate-buffered saline (PBS) to an equal volume of 3% formaldehyde followed by incubation for 18 hr at 37°C with agitation. The cells were washed five times in balanced salt solution (BSS) (12) and stored for 3 days in fetal calf serum. After washing in BSS, they were kept frozen at -20°C. G-CRBC were prepared by mixing 2 ml of 25% red cells with 25 ml of BSS and adding an equal amount of 0.25% glutaraldehyde for 5 min at 20°C. The cells were washed and treated as described for F-CRBC. Agglutination titrations were done, using a 0.5% red cell suspension in PBS and microtiter equipment (Microbiological Associates, Inc., Bethesda, Md.).

TABLE II  
*Release of Antigenic Material From Normal Vs. Fixed CRBC by Mφ*

Antigen in culture	pfc/10 <sup>6</sup> recovered cells on day 5
—	<0.2
10 <sup>6</sup> CRBC	2080
CRBC (supernatant)	290
F-CRBC (supernatant)	<0.2
Mφ (supernatant)	114
CRBC-Mφ (supernatant)	1280
F-CRBC-Mφ (supernatant)	<0.2

B6D2F<sub>1</sub> mice were immunized with 0.2 ml 10% CRBC on day -7. On day -3, they were injected intraperitoneally with 1.5 ml of proteose peptone (4); 10<sup>7</sup> PEC were incubated with or without 2 × 10<sup>8</sup> CRBC or F-CRBC in 2 ml of tissue culture medium (4) for 24 hr. The supernatants of these incubations were harvested by centrifugation (10 min at 1000 rpm) and 0.5-ml aliquots were mixed with 0.5 ml of fresh tissue culture medium and 1.3 × 10<sup>7</sup> spleen cells from B6D2F<sub>1</sub> mice primed with 0.2 ml 10% CRBC on day -30. Assays for pfc were done on day 5 (14).

CRBC supernatant = supernatant from CRBC incubated without Mφ; F-CRBC supernatant = supernatant from F-CRBC incubated without Mφ; Mφ supernatant = supernatant from Mφ incubated without red cells; CRBC-Mφ supernatant = supernatant from CRBC incubated with Mφ; F-CRBC-Mφ supernatant = supernatant from F-CRBC incubated with Mφ.

suspension of CRBC alone also stimulated an in vitro response, probably due to spontaneous lysis of cells, but the effect was much smaller. The same was true for the Mφ supernatant. In this case, it is possible that antigen had been

carried over by the PEC due to the prior intraperitoneal immunization on day -7. However, there was no release of immunogenic material from F-CRBC alone or if admixed with PEC, indicating that fixed cells cannot be processed by  $M\phi$  to a soluble immunogenic form. Because the  $M\phi$  supernatant by itself had some activity, it is somewhat surprising that the F-CRBC incubated with  $M\phi$  was completely inactive. We have no explanation for this finding. That the F-CRBC are simply toxic in in vitro cultures is excluded by the experiments referred to below (Table IV).

Mice injected with F-CRBC and G-CRBC showed a very poor primary response, if any, to these antigens as compared with the response to CRBC (Table III). But on challenge with CRBC, 7 days after the primary immuniza-

TABLE III  
*In Vivo Response to CRBC and Fixed CRBC*

Antigen per mouse	Primary response		Antigen per mouse	Secondary response	
	pfc/10 <sup>8</sup> spleen cells on day 5			pfc/10 <sup>8</sup> spleen cells on day 11	
	19S	19S + 7S		19S	19S + 7S
$1.5 \times 10^8$ CRBC	880	880	$1.5 \times 10^8$ CRBC	3168	16,068
$1.5 \times 10^8$ F-CRBC	118	30	$1.5 \times 10^8$ CRBC	4928	23,500
$1.5 \times 10^8$ G-CRBC	188	106	$1.5 \times 10^8$ CRBC	2726	17,400
—	139*	114*	—	—	—

Groups of 10 B6D2F<sub>1</sub> mice were intravenously injected, respectively, with  $1.5 \times 10^8$  CRBC, F-CRBC, and G-CRBC. On day 5, direct and direct plus indirect pfc were assayed in the spleens. 6 days after primary injection, all three groups were challenged with  $1.5 \times 10^8$  CRBC and the secondary response was assayed on day 11. Figures given indicate mean values of groups of five mice. Indirect plaques were developed by incubation with an appropriate dilution of a rabbit anti-mouse IgG serum 1 hr before adding guinea pig complement.

\* Background values represent upper limits of pooled determinations for 10 animals.

tion, all three groups gave very high 19S and 7S responses. It was striking that the animals primed with F-CRBC always showed somewhat higher responses than the controls injected with CRBC. This demonstrated that though the fixed red cells were not able to evoke a humoral response, they were in fact at least as potent as normal cells in priming the animals for a secondary response.

To find out whether either plaque-forming cell (pfc) precursors or thymus-derived helper cells had been primed, mice were injected with increasing doses of CRBC and F-CRBC. 5 days later, the spleens of these animals were cultured with trinitrophenyl (TNP)-CRBC (13), and the response to CRBC- and TNP-antigen was assayed separately. Fig. 1 shows that a dose of  $10^4$  CRBC gave optimal priming for both the CRBC and TNP response; lower and higher doses of antigen were somewhat less effective. This result which also holds for priming with SRBC in mice (reference 14 and our unpublished results) is quite different from that found with F-CRBC. Low doses of F-CRBC showed poorer priming, whereas high doses give much better priming than CRBC.

As the anti-TNP response was even somewhat better than the anti-CRBC response, it can be concluded that the thymus-derived helper cells and not the pfc precursors had been primed. Otherwise, only a stimulation of the anti-CRBC response would have been observed.

If this interpretation is correct, injection of thymus cells and fixed red cells into lethally irradiated animals should result in priming of thymus-derived helper cells (15). Table IV shows that T spleen cells sensitized to CRBC, F-CRBC, and G-CRBC stimulate both the anti-CRBC and anti-TNP responses equally well. But, as found in vivo, neither F-CRBC nor G-CRBC

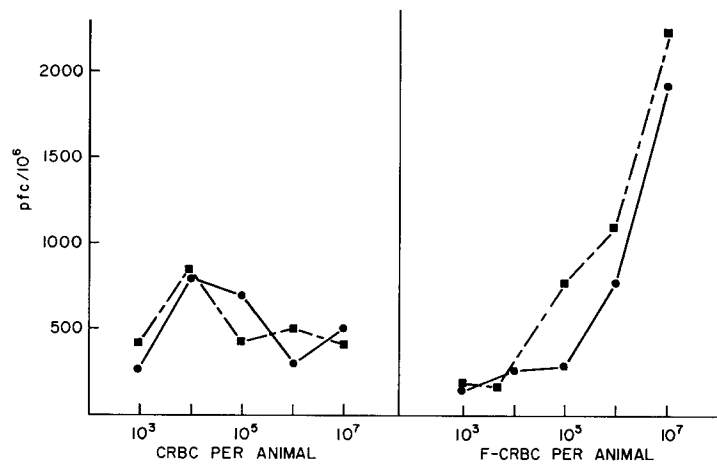


FIG. 1. Groups of three ♀ B6D2F<sub>1</sub> mice were injected intravenously with 10<sup>3</sup>–10<sup>7</sup> CRBC or F-CRBC. 5 days later spleens were cultured with 10<sup>6</sup> TNP-CRBC (13) in triplicate dishes for 5 days. Direct pfc were assayed on CRBC and TNP-burro erythrocytes (BRBC) and are expressed as pfc per 10<sup>6</sup> recovered cells. ●—●, anti-CRBC pfc; ■—■, anti-TNP pfc.

stimulated a pfc response. To exclude that the fixed red cells are toxic in in vitro cultures, they were mixed with TNP-CRBC. As can be seen from Table IV, a perfectly normal response to both CRBC and TNP antigens developed.

In summary, we have shown that fixed CRBC retain their immunogenicity toward T cells (Fig. 1, Table IV), but fail to induce the humoral response as measured in terms of numbers of pfc (Tables III and IV). Essentially similar results (not described here) have been obtained with fixed SRBC. Given in low doses, fixed cells seem to be slightly less effective than untreated red cells, but high doses show very good T cell priming (Fig. 1). M $\phi$  release soluble immunogenic material from normal red cells (Table II), but fail to do so from fixed red cells. Thus we show that chemical modification of the immunogen prevents M $\phi$  from releasing soluble immunogen, and also interferes in an asymmetrical way with B cell and T cell priming. While this may imply that antigen processing is a prerequisite for B cell priming, it does not exclude the participation of M $\phi$  in some other way in T cell priming.

These findings might provide a powerful tool for sensitizing T cells and raising cell-mediated immune responses to allogeneic and tumor-specific cell surface antigens. Preliminary experiments using such systems have, in fact, shown that fixed allogeneic and syngeneic tumor cells are able to raise cell-

TABLE IV  
*Priming of T Spleen Cells by CRBC, F-CRBC, and G-CRBC*

Sensitized T spleen cells per culture	Antigen per culture	pfc/10 <sup>6</sup> recovered cells on	
		CRBC	TNP-BRBC
—	—	4	3
—	10 <sup>6</sup> TNP-CRBC	6	30
—	10 <sup>6</sup> F-CRBC	<1	—
—	10 <sup>6</sup> G-CRBC	2	—
5 × 10 <sup>6</sup> T cells (CRBC)	—	1	1
5 × 10 <sup>6</sup> “ “	10 <sup>6</sup> TNP-CRBC	415	1310
5 × 10 <sup>6</sup> “ “	10 <sup>6</sup> F-CRBC	<1	—
5 × 10 <sup>6</sup> “ “	10 <sup>6</sup> G-CRBC	1	—
5 × 10 <sup>6</sup> T cells (CRBC)	10 <sup>6</sup> TNP-CRBC + 10 <sup>6</sup> F-CRBC	554	1790
5 × 10 <sup>6</sup> “ “	10 <sup>6</sup> TNP-CRBC + 10 <sup>6</sup> G-CRBC	800	1980
5 × 10 <sup>6</sup> T cells (F-CRBC)	—	1	23
5 × 10 <sup>6</sup> “ “	10 <sup>6</sup> TNP-CRBC	375	1180
5 × 10 <sup>6</sup> “ “	10 <sup>6</sup> F-CRBC	<1	4
5 × 10 <sup>6</sup> T cells (G-CRBC)	—	5	4
5 × 10 <sup>6</sup> “ “	10 <sup>6</sup> TNP-CRBC	310	950
5 × 10 <sup>6</sup> “ “	10 <sup>6</sup> G-CRBC	<1	—

10<sup>8</sup> thymus cells and 1.4 × 10<sup>8</sup> CRBC, F-CRBC, and G-CRBC suspensions were injected into lethally irradiated (1000 R, <sup>60</sup>Co) B6D2F<sub>1</sub> mice (14). 8 days later T spleens were harvested and 5 × 10<sup>6</sup> T spleen cells were mixed with 10<sup>7</sup> B spleen cells (spleen cells of thymectomized, lethally irradiated, and bone marrow-protected animals) per culture and antigen as indicated. Pfc were assayed on day 5.

T cells (CRBC) = T spleen cells sensitized with CRBC; T cells (F-CRBC) = T spleen cells sensitized with F-CRBC; T cells (G-CRBC) = T spleen cells sensitized with G-CRBC.

mediated immunity. Experiments are in progress to further exploit this technique.

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