COMPARATIVE ANALYSIS OF ANTIGEN-BINDING T CELLS IN GENETIC HIGH AND LOW RESPONDER MICE*

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The immune response to many antigens has been demonstrated to be under control of immune response (*Ir*) genes linked to the major histocompatibility complex of the species. The first *Ir* gene detected in mice is *Ir-1* (now designated *Ir-1A* [1]) which controls the humoral antibody response to the synthetic polypeptide (T,G)-A--L (2). Numerous studies on the mode of action of *Ir* genes have been performed, and, in the light of the presently available experimental evidence, it seems reasonable to assume that *Ir-1A*, and other *H-2*-linked *Ir* genes, are selectively expressed in thymus-derived (T) cells where they may code for or modify the still unknown T-cell receptor for antigen (3). However, conflicting evidence has been reported suggesting expression of *Ir-1A* in B-cells (4).

If *Ir-1A* is indeed a structural gene for the T-cell receptor, then one would expect to find a defect of low responder T-lymphocytes in their ability to interact with antigen. The present study shows that a difference in the frequency of [^{125}I](T,G)-A--L-binding T cells can be found only in immunized high and low responder mice, but not in unimmunized animals.

Materials and Methods

Mice. The following mice were bred at Stanford: C3H/DiSn (*H-2*^{k}, *Ir-1A*^{low}, *Ig*^{a}), C3H.SW (CSW) (*H-2*^{b}, *Ir-1A*^{high}, *Ig*^{a}), and C3H.WB/13 (CWB) (*H-2*^{b}, *Ir-1A*^{high}, *Ig*^{b}). In some instances C3H/HeJ mice were used instead of C3H/DiSn, with the same results.

Antigen-Binding Cells. Mice were immunized in the hind footpads with (T,G)-A--L 509 either in complete Freund's adjuvant (CFA)[1] or in phosphate-buffered saline (PBS). Highly purified T lymphocytes were obtained from popliteal and inguinal lymph nodes by elimination of B cells with nylon wool columns (5). These preparations were routinely tested in a cytotoxic assay with

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[1] *Abbreviations used in this paper:* Ars-MSA, arsanyl mouse serum albumin; ^{Ars}B-ABC, arsanyl-binding B cells; CFA, complete Freund's adjuvant; Ig, immunoglobulin allotype locus; PBS, phosphate-buffered saline; T-ABC, antigen-binding T cells.

anti-Thy-1.2 serum and were always found to be >90% Thy-1.2 positive. (T,G)-A--L-binding cells were prepared as previously described (6) by incubation of 5×10^6 cells in 0.2 ml medium for 20 min at 4 C with 0.3 μg [^{125}I](T,G)-A--L [6–10 μCi/μg = 1 molecule ^{125}I per (T,G)-A--L]. Arsanyl-specific ABC were prepared by incubation of 5×10^6 lymphocytes in 0.2 ml medium with 1 μg ^{125}I-labeled arsanylated mouse serum albumin [spec act 6 μCi/μg] in the presence of 5 μg free MSA. Excess radiolabeled antigen was washed off and cells were processed for autoradiography utilizing Kodak NTB-2 emulsion. Antigen-binding T cells (T-ABC) were detected in purified T-cell populations after 14-day exposure time of autoradiographs, whereas antigen-binding B cells were exclusively detected in unfractionated lymphocyte populations after 4-day autoradiographic exposure time (6). Only morphologically intact lymphocytes labeled with more than six grains per cell were scored as positive. Under the conditions used (0.3 μg (T,G)-A--L per incubation mixture, 14 days exposure time) T-ABC with about 200–500 molecules (T,G)-A--L bound per cell were detected (6).

Antisera. A.TH anti-A.TL serum (O13) was raised by injection of A.TL lymphocytes into A.TH mice. Another A.TH anti-A.TL serum (Shr) was a generous gift from Dr. D. C. Shreffler, University of Michigan, Ann Arbor, Mich. Anti-*H-2*^{k} serum was raised in CSW mice against C3H lymphocytes.

Results

Effect of Immunization on the Frequency of T-ABC. In a previous study, it was shown that [^{125}I](T,G)-A--L-binding cells detected in purified T-cell populations were indeed T-ABC (6). The numbers of T-ABC observed before and after immunization of high (CWB) and low (C3H) responder mice are depicted in Table I. It can be seen from this table that in unimmunized high and low responder animals approximately the same frequency of T-ABC was found. After immunization with (T,G)-A--L in CFA, both strains have the same slightly increased number of T-ABC. However, after secondary challenge with antigen, the number of T-ABC is increased only in high responder animals, but is unchanged or decreased in low responder mice.

In order to avoid any effects of CFA, immunization was also performed with

TABLE I

Influence of Immunization on Frequency of T-ABC in High and Low Responder Mice

Strain	Immunization	T-ABC/10^4	
		Mean*	Range
CWB	—	32	(27–35)
C3H	—	25	(19–30)
CWB	1°	50	(46–60)
C3H	1°	46	(43–53)
CWB	2°	84	(73–101)
C3H	2°	32	(20–39)

Two-four mice per group were sacrificed 21 days after immunization in the hind footpads with 10 μg (T,G)-A--L in CFA (1°) or challenged again with 10 μg (T,G)-A--L in saline, and sacrificed 10 days later. T-ABC were determined in purified T-cell populations from popliteal and inguinal lymph nodes.

* Mean of 3–5 experiments.

(T,G)-A--L in aqueous solution. The results are summarized in Fig. 1, which shows that only high responder T-ABC proliferate after immunization whereas the frequency of T-ABC in low responder mice remains unchanged.

Antigen Dose Dependence of T-ABC Frequency. The finding of similar numbers of T-ABC in unimmunized high and low responder mice was unexpected. Since ABC are produced with an excess of radiolabeled antigen, many ABC may be detected which have a very low avidity for the antigen, thus obscuring detection of potential avidity differences between high and low responder antigen-reactive T cells. Therefore, T-ABC were prepared with a range of concentrations of [^{125}I](T,G)-A--L. The resulting dose-response curves are shown in Fig. 2. In both strains, the frequency of T-ABC increases proportionally with the amount of antigen. Moreover, the dose-response curves for high and low

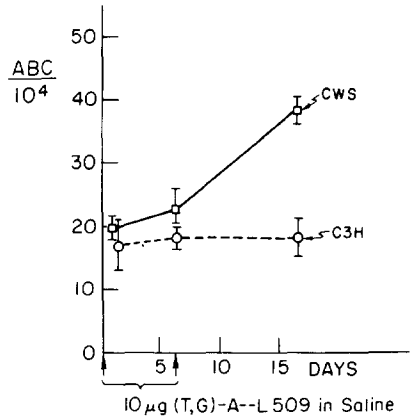

FIG. 1. Influence of immunization with (T,G)-A--L in aqueous solution on frequency of T-ABC. Six mice per group were sacrificed either 7 days after immunization in the hind footpads with 10 μg (T,G)-A--L in saline, or following a second challenge on day 7 and sacrifice 12 days later. Purified T cells were isolated from popliteal and inguinal lymph nodes. In this particular experiment, T-ABC were prepared by incubation of cells with 0.2 μg [^{125}I](T,G)-A--L per 0.2 ml incubation mixture.

responder mice are parallel, suggesting that, by this method, no avidity differences are detected. Note that C3H mice have regularly less T-ABC than CSW mice do. However, these differences are too small to serve as an argument for a T-cell defect of low responder mice.

Comparison of ABC in H-2 Linked and Allotype-Linked Immune Responses. In addition to *H-2*-linked *Ir* genes, which are expressed in T cells, there exists another class of *Ir* genes which are linked to the immunoglobulin allotype locus (Ig) and which probably act at the B-cell level, where they affect the structure of the immunoglobulin receptor for antigen. Therefore, a different pattern of antigen-binding B and T cells in *H-2*-linked and Ig-linked *Ir* systems might be expected. Pawlak et al. (7) described that the idiotype of anti-arsanyl antibody is linked to the immunoglobulin allotype, and S. Segal (personal communication) has shown recently that the antibody response to the arsanyl hapten is controlled by an allotype associated gene. C3H.SW (Ig^a) mice are high

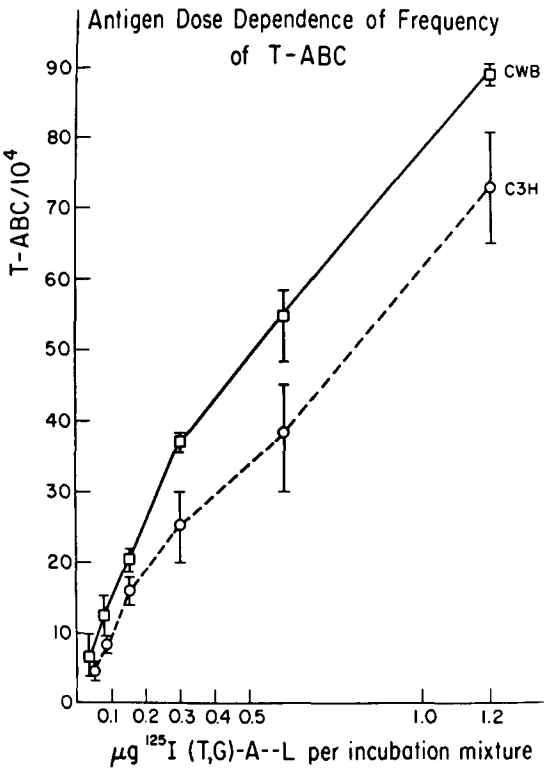

FIG. 2. Antigen dose dependence of frequency of T-ABC. Nonimmunized lymph node T cells isolated by nylon wool fractionation were incubated with increasing amounts of [^{125}I](T,G)-A--L and then processed for 14 days in autoradiography.

responders after immunization with arsanyl conjugated with keyhole limpet hemocyanin in CFA, whereas CWB (Ig^b) mice are low responders. In this study, arsanyl-binding T and B cells were detected with radiolabeled arsanyl mouse serum albumin conjugates (Ars-MSA) in the presence of an excess of free MSA. Details of preparation and specificity of arsanyl-binding cells will be described elsewhere. (It should be recalled that short autoradiographic exposure time [4 days] permits the selective detection of B-ABC in unfractionated cell populations [1, 8].) Fig. 3 shows the results when T- and B-ABC in the (T, G)-A--L and Ars-MSA systems were compared. It can be seen that after secondary immunization many more arsanyl-binding B cells (^{Ars}B-ABC) are found in the high responder strain (CSW) than in the low responder strain. On the other hand, ^{Ars}T-ABC proliferate to the same extent in both CSW and CWB mice. Taken together, these results indicate a B-cell defect in the Ig-linked *Ir* system and a T-cell defect in the *H-2*-linked *Ir-1A* system.

Failure of Anti-Ia Sera to Inhibit ABC. In a previous communication (9) it was reported that anti-*H-2* sera completely block T-ABC, but not B-ABC. It was not clear whether the inhibition was caused by anti-*H-2* antibodies or by a contaminating antibody population specific for *Ir*-gene products. Recently, antisera became available which were directed exclusively against I-region

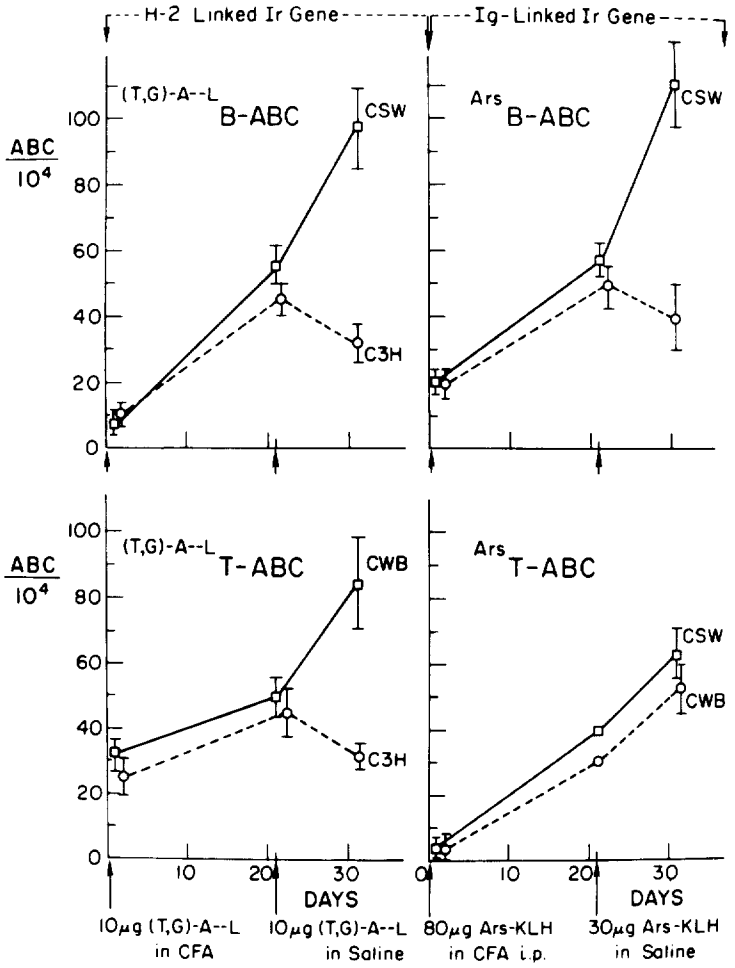

FIG. 3. Comparison of B-ABC and T-ABC in *H-2* controlled and immunoglobulin allotype (*Ig*) linked *Ir* systems. B-ABC were selectively detected in unfractionated cell suspensions utilizing 4-day exposure times of autoradiographs, whereas, for T-ABC, purified T cells and 14-day exposure times were used. (T,G)-A--L specific ABC were produced in lymph node suspensions after immunization of mice with (T,G)-A--L in the hind footpads as indicated in Fig. 3. Arsanyl specific ABC were prepared by incubation of splenic lymphocytes with 1 μg ^{125}I-labeled arsanylated mouse serum albumin in the presence of excess free MSA. For immunization, mice were challenged i.p. with 80 μg Ars-keyhole limpet hemocyanin (Ars-KLH) in CFA. For secondary challenge, 30 μg Ars-KLH in saline was injected i.p. It should be noted that comparable results were obtained when Ars-KLH was administered in the hind footpad and ABC were determined in draining lymph nodes.

products (Ia-antigens) without displaying any activity against *H-2K* or *H-2D* determinants (10–12). These anti-Ia sera were shown to react predominantly with B lymphocytes (12). Two different anti-Ia sera (A.TH anti-A.TL) were tested for their ability to block ABC. Since A.TL and C3H mice have identical or very similar *I* regions (both strains have I^k), A.TH anti-A.TL sera react with the majority of Ia antigens on C3H cells, and since A.TL and CSW have some Ia

specificities in common, a reaction with CSW cells has also been observed (21). One serum (013) was directed exclusively against B cells (12), whereas the other serum (Shr) also contained some T-cell cytotoxicity (10). Table II shows that neither serum inhibited T- or B-ABC, suggesting the absence of antibodies against T- or B-cell receptors. In contrast, when anti-*H-2*^{k} serum was included as a positive control, blocking of T-ABC was observed as described previously (9).

Discussion

ABC are usually studied under conditions which give a reasonable (but arbitrary) number of ABC. The proportional increase in the frequency of T-ABC with

TABLE II

Failure of Anti-Ia Sera to Inhibit [^{125}I](*T,G*)-*A--L Binding T and B Cells**

Strain	Immunization	A.TH Anti-A.TL Serum	Dilution	T-ABC		B-ABC	
				ABC/10^4	Inhibition	ABC/10^4	Inhibition
					%		%
C3H	Nonimmune	—	—	26	—	ND	
		013	1:5	21	20	ND	
		Shr	1:5	29	0	ND	
C3H	Immune‡	—	—	42	—	53	—
		013	1:5	52	0	53	0
		013	1:10	29	32	49	10
		013	1:20	44	0	57	0
		Shr	1:5	40	5	ND	
		Shr	1:10	39	8	ND	
		Anti-*H-2*^{k}	1:5	6	86	50	6
		Anti-*H-2*^{k}	1:10	4	90	ND	
CSW	Immune‡	—	—	48	—	55	—
		013	1:5	51	0	48	13

*B-ABC were selectively detected in unfractionated lymph node populations after short (4 day) exposure times of autoradiographs. T-ABC were detected in purified T-cell populations after 14 days of autoradiography.

‡Popliteal and inguinal lymph nodes were taken from mice 3 wk after challenge with 10 μg (T,G)-A--L in CFA in the hind footpads.

antigen concentration, and the relatively high number of T-ABC, suggest that only a small percentage of T-ABC belong to the pool of truly specific immunocompetent cells. Even at a concentration of 1.2 μg [^{125}I](T,G)-A--L per 0.2 ml, no plateau for T-ABC was observed. Roelants and Ryden (13), who reported comparable results, obtained maxima for T-ABC only when more than 5 μg lightly labeled (T,G)-A--L was used. Because of the possibility that T cells bind antigen by means of cytophilic B-cell derived antibody, it is possible that the majority of T-ABC are artifactual. Our previous studies showing marked differences in the specificity of T- and B-ABC for (T,G)-A--L (6), and the present

observation (demonstrating that, in the *Ig*-linked anti-Ars response, low responder ^{Ars}T-ABC proliferate, although the number of ^{Ars}B-ABC decreases), render it unlikely that all T-ABC are artifacts. Therefore, it is reasonable to assume that T-ABC and B-ABC partly reflect the true immune status of the animal, although the functional activity of T-ABC has not yet been shown. If this assumption is accepted, two main conclusions can be drawn: (*a*) Although it is generally believed that immunoglobulin allotype-linked *Ir* genes act at the B-cell level, direct experimental proof has been lacking. The present study provides experimental support for this hypothesis, and also indicates that immunoglobulin allotype linked-*Ir* genes are not expressed in T cells; (*b*) The failure of T-ABC to proliferate in the *Ir-1A* gene controlled low responder strain is similar to the lack of in vitro stimulation by antigen of *Ir-1A* low responder T cells described by P. Lonai (14). Thus, these results support the hypothesis that *Ir-1A* is expressed at the T-cell level, and are in contrast to reports suggesting an exclusive defect in low responder B cells (4). The reason for this discrepancy is not clear. The present study does not exclude an additional defect in low responder B cells. However, the lack of proliferation of low responder B cells may well be explained by the relative lack of an appropriate T-cell population.

There are two possibilities for the failure to detect avidity differences between high and low responder T cells. First, it could be that an avidity difference does not exist, implying that *Ir-1A* does not code for or influence the T-cell receptor, but controls an event taking place after the antigen is bound to the receptor. In this case, it is difficult to understand how the effect of *Ir-1A* can be so highly antigen specific. Second, the method of demonstrating avidity differences of cellular receptors for a multivalent antigen such as (T,G)-A--L may not be sufficiently sensitive. Moreover, if only a small proportion of T-ABC belongs to the truly immunocompetent cell pool, then avidity differences might be difficult to detect. Since there is evidence that the affinity of T-ABC does not change significantly after immunization (13, 15) one might expect a difference in the number of T-ABC in high and low responder strains, as was found in immunized and unimmunized high responder mice. Because of the inadequacy of our present experimental techniques, these questions must remain unresolved. In addition, the failure of anti-Ia to block T-ABC cannot be used as an argument against the possibility that *Ir-1A* codes for the T-cell receptor. A more likely explanation is that anti-Ia sera used in this study do not contain antibodies against an *Ir-1A* gene product. However, since different preparations of anti-Ia sera appear to vary considerably with respect to their activity and cellular specificity (10–12), a large variety of anti-Ia sera has to be investigated in inhibition studies.

Summary

[^{125}I](T,G)-A--L-binding T cells have been studied in mice whose ability to mount an immune response to (T,G)-A--L is under control of the *H-2*-linked *Ir-1A* gene. Nonimmunized high and low responder mice have approximately the same frequency of T-ABC. Following immunization, T-ABC proliferated only in

high responders, but not in low responders, indicating expression of *Ir-1A* in T cells. When, for comparison, [^{125}I]arsanyl-mouse serum albumin binding B and T cells were investigated in mice whose antibody response to the hapten arsanyl is controlled by an allotype-linked *Ir* gene, it was found that following immunization the number of B-ABC increased only in high responders. In contrast, T-ABC proliferated to the same extent in both high and low responders, suggesting exclusive expression of the allotype-linked *Ir* gene in the B-cell line. Preliminary studies indicate that anti-Ia sera inhibit neither B-ABC nor T-ABC.

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