

ANTIGEN-SPECIFIC T-CELL FACTORS IN THE GENETIC
CONTROL OF THE IMMUNE RESPONSE TO
POLY(TYR, GLU)-POLYDLALA--POLYLYS

Evidence for T- and B-Cell Defects in SJL Mice*

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We have previously reported experiments in which an antigen-specific T-cell "factor" was utilized to directly study the role of different cell types in the genetic control of the immune response in mice to the synthetic polypeptide poly(Tyr, Glu)-polyDLAla--polyLys [(T,G)-A--L] (1, 2). The T-cell factor is obtained from educated thymocytes and is capable of cooperating with bone marrow (B) cells in irradiated recipients (3). In our previous work, educated T cells of high responder (C3H.SW, *H-2^b*) and low responder (C3H/HeJ, *H-2^k*) origins were compared for their ability to produce the T-cell factor, and B cells of the two strains were compared for their ability to produce antibody in response to T-cell factor and antigen. It was found that educated T cells of both high and low responder strains were equally efficient at producing the cooperative T-cell factor, but that B cells of high responder origin only were able to cooperate with the factor and antigen in vivo (1, 2). These results provided direct evidence that the genetic defect in the low responder (*H-2^k*) strain was not reflected in T-cell function, but was rather associated with the low responder B cells. The same conclusion has been drawn from limiting dilution experiments (4). We have now extended these studies to another low responder strain, SJL/J (*H-2^s*). The results suggest that in this case both T and B cells are defective in their ability to respond to (T,G)-A--L.

Materials and Methods

Antigens. The following multichain synthetic polypeptides were used: (a) Poly(LTyr, LGlu)-PolyDLAla--polyLys, abbreviated as (T,G)-A--L, batch no. 1383. (b) Poly(LTyr, LGlu)-polyLPro--polyLys, abbreviated as (T,G)-Pro--L, batch no. 935. The synthesis and characterization of such immunogens have been previously described (5, 6).

Animals. Mice of the strains C3H.SW (*H-2^b*) and SJL/J (*H-2^s*), bred at The Experimental Animal Unit, The Weizmann Institute of Science, Rehovot, Israel, were used.

Preparation and Test of T-Cell Factors. The methods used for the preparation of T-cell factors

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from educated T cells in vitro and their test with B cells in vivo were as described in detail in previous papers (1, 3).

Hemolytic Plaque-Forming Cell Assay. Direct plaque forming cells (PFC) in the spleens of recipient mice were determined using (T,G)-Pro-L-coated sheep red blood cells, as previously described (1).

Results

Activity of T-Cell Factors of High and Low Responder Strains. In the first experiments, T-cell factors for (T,G)-A--L were prepared from (T,G)-A--L-educated T cells of high responder (C3H.SW) and low responder (SJL) origin. The factors were tested for their ability to cooperate with B cells of high responder origin, by transfer together with C3H.SW B cells and (T,G)-A--L into irradiated C3H.SW recipients. The upper part of Table I shows that whereas C3H.SW factor cooperated efficiently with C3H.SW B cells, low responder SJL

TABLE I
Ability of T-Cell Factors Produced by C3H.SW and SJL T Cells to Cooperate with C3H.SW B Cells

Cells and factors transferred into irradiated recipients				Mean PFC per spleen*		
B cells‡	T cells§	Factor	Antigen¶	Log ₁₀	Standard error	Antilog
C3H.SW	—	—	(T,G)-A--L	2.908	0.020	810
C3H.SW	C3H.SW	—	(T,G)-A--L	5.010	0.022	102,400
C3H.SW	—	C3H.SW	(T,G)-A--L	4.980	0.020	95,665
C3H.SW	—	SJL	(T,G)-A--L	2.418	0.054	262
C3H.SW	—	—	(T,G)-Pro--L	3.244	0.035	1753
C3H.SW	C3H.SW	—	(T,G)-Pro--L	4.951	0.026	89,410
C3H.SW	—	C3H.SW	(T,G)-Pro--L	4.969	0.022	93,139
C3H.SW	—	SJL	(T,G)-Pro--L	4.970	0.016	93,368

* Geometric means of direct PFC 12 days after transfer.

‡ 10⁷ Bone marrow.

§ 10⁸ thymocytes.

|| Spleen equivalent.

¶ 10 μg in saline.

factor totally failed to collaborate in the response of these B cells to (T,G)-A--L. The number of plaques produced by C3H.SW B cells and SJL factor did not rise above the background.

This result could imply either an incompetence on the part of SJL cells to produce cooperative factor for (T,G)-A--L; or alternatively reflect a more general inability of factors produced by the SJL (*H-2^s*) strain to collaborate with C3H.SW (*H-2^b*) B cells. To distinguish these possibilities, control experiments were set up in which T-cell factors were produced in the same two strains, C3H.SW and SJL, to another antigen, (T,G)-Pro--L, to which both strains are high responders. The lower part of Table I shows that in this case, both C3H.SW and SJL T-cell factors cooperated with equal efficiency with C3H.SW B cells. Therefore, it seems that there is a specific inability of SJL-educated T cells to produce a cooperative factor for (T,G)-A--L.

Activity of B Cells of Low Responder Strains. B cells of the low responder SJL strain were tested for their responsiveness to T-cell factors, for (T,G)-A--L,

produced either syngeneically or by the high responder strain, C3H.SW-educated T cells. As shown in Table II (upper part) SJL B cells failed to generate an antibody response to (T,G)-A--L when combined with T-cell factors of either syngeneic or high responder strains. Moreover, the lower part of Table II shows that there was no defect in cooperation per se between C3H.SW factor and SJL B cells, using the response to (T,G)-Pro--L as a positive control. Thus, since the C3H.SW factor for (T,G)-A--L is active in cooperation (Table I), it appears that SJL B cells are unable to mount an antibody response to (T,G)-A--L. Taken together with the results above, it is concluded that the defect in response to (T,G)-A--L in SJL mice is present in both T- and B-cell populations.

TABLE II
Ability of SJL B Cells to Cooperate with T-Cell Factors Produced by SJL and C3H.SW T Cells

Cells and factors transferred into irradiated recipients				Mean PFC per spleen*		
B cells‡	T cells§	Factor	Antigen¶	Log ₁₀	Standard error	Antilog
SJL	—	—	(T,G)-A--L	.2099	0.045	126
SJL	SJL	—	(T,G)-A--L	2.376	0.066	237
SJL	—	SJL	(T,G)-A--L	2.354	0.041	226
SJL	—	C3H.SW	(T,G)-A--L	2.361	0.055	229
SJL	—	—	(T,G)-Pro--L	2.294	0.056	197
SJL	SJL	—	(T,G)-Pro--L	4.956	0.030	90,407
SJL	—	SJL	(T,G)-Pro--L	4.997	0.039	99,430
SJL	—	C3H.SW	(T,G)-Pro--L	4.953	0.016	89,848

* Geometric means of direct PFC 12 days after transfer.

‡ 10⁷ bone marrow.

§ 10⁸ thymocytes.

|| Spleen equivalent.

¶ 10 μg in saline.

Discussion

In the present study we have used an antigen-specific T-cell product, or factors, to probe the cellular basis of genetic control of the antibody response to (T,G)-A--L in SJL (low responder) mice. The major characteristics of the T-cell factor used are its specificity for antigen (3, 7), its ability to replace T cells in thymus-dependent antibody responses (1, 3), and its nature as a product of the major histocompatibility (*H-2*) complex in the mouse (7, 8). An important advantage of using the T-cell factor in this work, rather than T cells themselves, is that allogeneic combinations of T-cell factor and B cells are generally as efficient, in terms of antibody production, as syngeneic combinations (1, 8), which is often not the case for allogeneic T- B-cell mixtures (9). Moreover, there is no evidence for any nonspecific "allogeneic" effects which frequently accompany the use of living T cells in allogeneic combination with B cells (10). Production of, and response to, the T-cell factors by the T and B cells of different strains can, therefore, be used as a direct gauge of T- and B-cell competence.

Previously, we have used T-cell factors to investigate the genetic defect in response to (T,G)-A--L in mice of the low responder C3H/HeJ (*H-2^k*) strain (1, 2). Comparison with high responder, C3H.SW (*H-2^b*) mice showed that the T

cells of the two strains were equally efficient in production of the (T,G)-A--L-specific cooperative T-cell factor. However, B cells of high responder origin only were able to mount an antibody response to (T,G)-A--L when stimulated by antigen and T-cell factor of either strain. In the case of the *H-2^k* strain, therefore, the defect in responsiveness to (T,G)-A--L resides specifically in the B-cell population. In contrast, in our present work, T and B cells of a different low responder strain and *H-2* haplotype, namely SJL (*H-2^s*), have been shown respectively to be incapable of either producing or responding to T-cell factor. Control experiments confirmed that SJL mice both produce and respond to T-cell factors for another antigen, (T,G)-Pro--L, to which they are high responders (Tables I and II). Thus, we conclude that SJL mice carry an antigen-specific defect which is reflected in the responsiveness of both T- and B-cell populations. In this respect, these results are in agreement with those of limiting dilution assay and allogeneic cell transfer (4). Since SJL mice are incapable of good antibody responses to any determinant attached to the A--L carrier, these results also support the hypothesis that defects in carrier function will be associated with helper T-cell populations (11).

It is noteworthy that although in these experiments only 19S (direct) PFC have been measured, previous work using C3H.SW and C3H/HeJ strain has shown that both 19S and 7S antibody production can be triggered by the T-cell factor under conditions of cell transfer (E. Mozes, personal observation). Furthermore, in this system, the genetic defect in low responder mice is expressed equally in both 19S and 7S antibody production.

Immune responsiveness to (T,G)-A--L, and other multichain synthetic polypeptides built on A--L, is controlled by the *Ir-1* gene which is itself a part of the *H-2* complex (12). Histocompatibility-linked *Ir*-gene function appears to be closely associated with various stages in cell cooperation (1, 13). We have previously suggested that the cellular defect in C3H/HeJ (*H-2^k*) low responders lies in the ability of the (T,G)-A--L-specific B cells to receive and respond to a T-cell signal imparted by the soluble T-cell factor (1). The defect in SJL mice is found in both T and B cells. One possible explanation is that cell cooperation is required in both cell lines, i.e. T-T as well as T-B cooperation, and that in SJL mice both T and B cells are unable to receive the T-cell signal. Alternatively, T cells in SJL mice may lack the antigen receptor for (T,G)-A--L, the specificity of which could be controlled by the *Ir-1* genes, while the SJL B cells are unable to receive the T-cell product.

Summary

The cellular basis of the genetic control of the immune response to poly(LTyr, LGlu)-polyDLA1a--polyLys [(T,G)-A--L] in SJL (*H-2^s*, low responder) mice has been investigated using T-cell factors. Thymocytes of SJL origin were educated to (T,G)-A--L and tested for their ability to produce an antigen-specific factor capable of cooperating in vivo with bone marrow cells of either SJL or C3H.SW (high responder) origin. SJL T cells were found to be incapable of producing such a cooperative factor, in contrast with results previously obtained with C3H/HeJ (low responders) and C3H.SW strains. Moreover, SJL bone marrow cells did not produce an antibody response to (T,G)-A--L, even when combined with factor

produced by high responder (C3H.SW) mice. Thus, both T and B cells appear to be defective in the SJL strain in the response to (T,G)-A--L.

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