Brief Definitive Report

ELICITATION OF DELAYED-TYPE HYPERSENSITIVITY RESPONSES TO poly(LTyr,LGlu)-poly(DLAla)--poly(LLys) BY ANTI-IDIOTYPIC ANTIBODIES*

By GIDEON STRASSMANN, RUTH LIFSHITZ, AND EDNA MOZES

From the Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel

The determination of biological activities of anti-idiotypic (Id) antibodies is a useful approach for studying antigen-specific T cell recognition unit because cross-reactive Id determinants have been shown to be shared by antibodies and T cells (1, 2). Anti-Id antibodies were reported to trigger Id-specific T cells that suppress antibody formation (3) and delayed-type hypersensitivity (DTH) responses (4). Helper and mixed-lymphocytic-reactive T cells were also reported to be induced by anti-Id antibodies (2, 5). Recently, we have reported that anti-Id serum produced in C57BL/6 mice against C3H.SW anti-poly(LTyr,LGlu)-poly(DLAla)--poly(LLys) [(T,G)-A--L] antibodies stimulated in vitro proliferative responses of (T,G)-A--L-primed T cells (6). Furthermore, anti-Id sera against (T,G)-A--L-specific antibodies (7) reacted with (T,G)-A--L-specific helper factors produced by educated T cells (8), a T cell-specific hybrid line, and a (T,G)-A--L-specific continuous line with helper activity (9).

DTH responses to (T,G)-A--L are T cell mediated, antigen specific (10), and genetically controlled (11). In a previous article we have described the participation of two distinct T cell subsets in DTH to (T,G)-A--L (12). We have shown that sensitized radioresistant Lyt-$1^+2^-3^-$ cells required the presence of normal radiosensitive Lyt-$1^+2^+3^+$ cells for efficient DTH responses. It was of interest to establish the effect of murine anti-Id serum against (T,G)-A--L-specific antibodies on T cell-mediated DTH responses. In this report we describe the ability of this anti-Id serum to replace the antigenic challenge in the efferent phase of DTH. We were able to localize the effect of the antiserum on the antigen-educated Lyt-$1^+2^-3^-$ cells.

Materials and Methods

Animals. C3H.SW (H-2^b, Igh-1^a), C57BL/6 (H-2^b, Igh-1^b), and CWB (H-2^b, Igh-1^b) mouse strains 2–3 mo of age were obtained from the Experimental Animal Unit of The Weizmann Institute of Science, Rehovot, Israel.

Antigens. The synthetic polypeptide (T,G)-A--L was synthesized and characterized as described previously (13). Keyhole lymphet hemocyanin (KLH; Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) was used as well.

Preparation of Anti-Id-Serum. Anti-(T,G)-A--L Id serum was produced in C57BL/6 mice. Briefly, mice were injected intravenously and subcutaneously with 50 μg of C3H.SW anti-(T,G)-A--L antibodies in complete Freund's adjuvant (CFA; H37Ra; Difco Laboratories, Detroit, Mich.). 1 wk later, the mice were injected with incomplete Freund's adjuvant, and boosted weekly thereafter, (total of six times) with antibodies in phosphate-buffered saline

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(PBS; 0.01 M phosphate buffer, pH 7.2, in 0.15 M NaCl). The serum was tested for idiotype-binding capacity by radioimmunoassay.

In Vivo Generation of Educated T Cells and Measurement of DTH. Thymocytes (10^8) were injected intravenously into irradiated (800 rad; Co source) syngeneic recipients that were immunized with 20 μg of antigen in CFA intraperitoneally. Spleens that contained educated T cells were removed 7 d later and single cell suspensions were prepared. Cells were irradiated (1,200 rad) and transferred into naive recipients. 16 h after cell transfer, mice were challenged with 10 μl of either antigen [(T,G)-A--L or KLH] (2 mg/ml) or with anti-Id serum diluted in PBS in the right ear (R). The left ear (L) was injected with either PBS (as control for antigen) or with C57BL/6 normal mouse serum (NMS) at the same dilution of anti-Id (as control for antiserum). 10 h after challenge, mice received 5-fluorodeoxyuridine and 2 μCi of [^{125}I]5-iodo-2′-deoxyuridine ([^{125}I]UdR; Radiochemical Centre, Amersham, England). Ears were removed 25 h later and counted in a gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The results are expressed as the ratio of radioactivity in the right ear to that of the left ear (R:L [^{125}I]UdR index; [10, 11]). Positive DTH was considered when the index was >1.2. The results are expressed as the arithmetic mean of all mice in the group ± SE. *P* values were calculated by the Student's *t* test.

Results

Effect of Anti-Id Serum on DTH to (T,G)-A--L. To find out whether anti-Id serum would have any effect on DTH to (T,G)-A--L, various dilutions of anti-Id serum were injected into the right pinnea of recipients that were transferred previously with (T, G)-A--L-educated and irradiated T cells. As a control, the same recipients were challenged with NMS in the left pinnea. As can be seen in Table I, the anti-Id could replace the antigenic challenge in the ears. Significant DTH responses could be observed when the anti-Id serum was injected at 1:100 and 1:200 dilutions. It can also be seen in Table I that no biological effect could be obtained in naive mice that did not receive (T,G)-A--L-activated cells and were challenged with the anti-Id serum at either 1:20 or 1:100 dilutions. No effect of the C57BL/6 anti-Id serum produced against C3H.SW anti-(T,G)-A--L antibodies could be observed on 20 × 10^6 KLH-educated and irradiated cells transferred into C3H.SW recipients (Table I). Thus, it can be concluded that the anti-Id serum can trigger DTH responses in mice transferred with (T,G)-A--L-activated cells of C3H.SW origin, and that this potential is antigen specific.

Strain Specificity of the Effect of the Anti-Id on DTH Responses to (T,G)-A--L. Table II

TABLE I

Dose-dependence and Specificity of the Effect of Anti-Id on DTH Responses to (T,G)-A--L

Group	Educated cells transferred*	Sensitizing antigen	Antigen used for ear challenge	Dilution of anti-Id used for challenge	Responders/ group	R:L [^{125}I]UdR index ± SE
A	25 × 10^6	(T,G)-A--L	(T,G)-A--L	—	9/10	1.44 ± 0.06‡
B	25 × 10^6	(T,G)-A--L	—	1:20	2/5	1.11 ± 0.09
C	25 × 10^6	(T,G)-A--L	—	1:100	10/11	1.42 ± 0.07§
D	25 × 10^6	(T,G)-A--L	—	1:200	8/10	1.37 ± 0.08‖
E	25 × 10^6	(T,G)-A--L	—	1:500	2/5	1.14 ± 0.10
F	—	—	—	1:20	1/6	1.05 ± 0.08
G	—	—	—	1:100	0/6	0.81 ± 0.07
H	20 × 10^6	KLH	KLH	—	9/10	1.80 ± 0.12¶
I	20 × 10^6	KLH	—	1:100	1/10	1.10 ± 0.06

* C3H.SW educated and irradiated (1,200 rad) cells were transferred into syngeneic recipients.
‡ Significant difference from group F: $P < 0.001$.
§ Significant difference from group F: $P < 0.002$.
‖ Significant difference from group F: $0.02 < P < 0.01$.
¶ Significant difference between groups H and I: $P < 0.001$.

TABLE II

Strain-specific Effect of the Anti-Id Serum on (T,G)-A--L-specific DTH Responses

Group	Mouse strain*	Allotype	Reagent used for challenge	Responders/group	R:L [^{125}I]UdR index ± SE
A	C3H.SW	Igh-1^a	(T,G)-A--L	8/10	1.45 ± 0.08‡
B	C3H.SW	Igh-1^a	Anti-Id§	13/15	1.40 ± 0.07‡
C	CWB	Igh-1^b	(T,G)-A--L	5/5	1.54 ± 0.05‖
D	CWB	Igh-1^b	Anti-Id§	1/7	1.05 ± 0.06
E	C57BL/6	Igh-1^b	(T,G)-A--L	10/10	1.68 ± 0.14‡
F	C57BL/6	Igh-1^b	Anti-Id§	1/10	0.96 ± 0.06

* 25 × 10^6 (T,G)-A--L-educated and irradiated (1,200 rad) cells were transferred into syngeneic naive recipients.

‡ Significant difference from group D: $P < 0.005$.

§ Dilution of anti-Id used for challenge was 1:100 in PBS.

‖ Significant difference from group D: $P < 0.001$.

TABLE III

Determination of the Cell Type Affected by the Anti-Id Antibodies

Educated cell donors*	Recipient strain	Challenging reagent	Responders/group	R:L [^{125}I]UdR index ± SE
C3H.SW	C57BL/6	(T,G)-A--L	13/16	1.70 ± 0.18‡
C57BL/6	C3H.SW	(T,G)-A--L	15/15	1.55 ± 0.12‡
C3H.SW	C57BL/6	Anti-Id§	15/17	1.51 ± 0.08‡
C57BL/6	C3H.SW	Anti-Id§	1/18	1.07 ± 0.07

* 25 × 10^6 (T,G)-A--L-educated T cells were irradiated (1,200 rad) and transferred into naive recipients.

§ Dilution of anti-Id serum used for challenge was 1:100 in PBS.

‡ Significant difference from the last group in the Table: $P < 0.01$.

demonstrates that the activity of the anti-Id serum on DTH responses is strain specific. Thus, the anti-Id serum replaces (T,G)-A--L in eliciting DTH responses only in C3H.SW (Igh-1^a) mice but not in CWB mice, which are congenic with C3H.SW and differ only by heavy-chain allotypes (Igh-1^b). C57BL/6-educated cells used as control were not triggered as well by the anti-Id serum (Table II). These results suggest allotype-linked cross-reactive idiotypic determinants between C3H.SW (T,G)-A--L-specific antibodies and DTH-mediating T cells.

Stimulatory Effect of the Anti-Id on the (T,G)-A--L-educated but Not on the Proliferating T Cells in DTH Responses. Efficient DTH responses require educated radioresistant Lyt-1$^+$2$^-$3$^-$ cells and normal radiosensitive Lyt-1$^+$2$^+$3$^+$ cells (12). It was of interest, therefore, to find out which cell type of the above-mentioned populations is triggered by the anti-Id. Because the anti-Id was shown to elicit DTH responses only in C3H.SW mice (H-2^b, Id$^+$) and not in C57BL/6 mice (H-2^b, Id$^-$) we have performed experiments in which C3H.SW educated T cells were transferred into C57BL/6 recipients, and vice versa, C57BL/6 educated cells were transferred into recipients of the second mouse strain. Recipients were challenged in the ear either with (T,G)-A--L or with the anti-Id. Table III demonstrates that educated T cells of C3H.SW origin can be triggered by (T,G)-A--L to mediate DTH responses in C57BL/6 naive recipients and vice versa as result of H-2^b compatibility. On the other hand, when the anti-Id (1:100) serum was used for ear challenge, DTH responses were obtained only when educated and irradiated T cells of C3H.SW origin were transferred into C57BL/6 naive recipients but not when educated C57BL/6 cells were injected into C3H.SW

recipients. Thus, the (T,G)-A--L-educated T cells are those triggered by the anti-Id in the efferent phase of the DTH response.

Discussion

In this study we have demonstrated the effectiveness of anti-Id in eliciting DTH responses mediated by (T,G)-A--L-educated T cells (Table I). This in vivo biological function of anti-Id on C3H.SW Id-positive educated cells is shown to be antigen (Table I) and strain (Table II) specific. The fact that CWB responder mice to (T,G)-A--L could not be triggered by the anti-Id to manifest DTH responses suggested a linkage between the expressed Id determinants on DTH-mediating T cells and the Igh-1^a allotypic marker of C3H.SW strain (Table II). These results are in agreement with previous data indicating a linkage between the heavy-chain allotypes and the expression of Id determinants on anti-(T,G)-A--L antibodies (14) and on (T,G)-A--L-specific helper T cell factor (8). With the same C57BL/6 anti-Id, shared Id determinants have been shown between subpopulations of T cells of different immune functions. Hence, the anti-Id reacted with (T,G)-A--L-specific helper factors (8, 9), it stimulated in vitro proliferating T cells (6), and here we have shown its capacity to challenge DTH-mediating T cells (Tables I and II).

The triggering effect of the anti-Id has been obtained only when the antigen-educated cells were originated from an Id^+ (C3H.SW) mouse strain, whereas the proliferating normal T cells participating in the efferent phase of the DTH response could be of an Id^- origin (Table II). These results contribute to the understanding of the mechanism of DTH reaction. It is likely that (T,G)-A--L, when used for ear challenge, triggers the antigen-activated T cell (Lyt-$1^+2^-3^-$). The latter, as a result, signals the second nonstimulated T cell (Lyt-$1^+2^+3^+$) to respond in the efferent phase of DTH.

Summary

The in vivo effect of murine anti-idiotypic serum against C3H.SW anti-poly(LTyr,LGlu)-poly(DLAla)--poly(LLys) [(T,G)-A--L] antibodies on delayed type hypersensitivity responses to (T,G)-A--L was studied. Anti-idiotypic serum could challenge DTH responses in C3H.SW mice transferred with antigen-sensitized T cells. The elicitation activity was shown to be antigen and strain specific. With H-2-compatible (but allotype different) strain combinations of (T,G)-A--L-educated T cells and recipients, we were able to show that the biological effect of the anti-idiotypic serum is expressed on the first antigen-sensitized idiotype-positive radioresistant T cell, but not on the proliferating normal cells of recipient origin that participate in the efferent phase of delayed-type hypersensitivity responses to (T,G)-A--L.

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