Brief Definitive Report

INDUCTION OF DELAYED-TYPE HYPERSENSITIVITY TO AZOBENZENEARSONATE BY A MONOCLONAL ANTI-IDIOTYPE ANTIBODY*

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Various T cell activities can be induced by anti-idiotypic antibodies, for example, helper and suppressor T cells for humoral immunity (1), and T cells mediating (2) or suppressing (3, 4) delayed-type hypersensitivity (DTH). Rabbit antibodies directed against the major cross-reactive idiotype (CRI) of antiazobenzenearsonate (ABA) antibodies of A/J mice can induce DTH in doses as low as 0.01 μg idiotype-binding capacity (2). We recently produced a monoclonal reagent, 14A, directed toward an idiotype located at or close to the combining site of a CRI^+ monoclonal anti-ABA antibody, 7.1.3 (5). We demonstrate in this paper that this idiotype is expressed by some ABA-specific T cells by using 14A to induce ABA-specific DTH in A/J mice.

Materials and Methods

Mice. 8–10-wk-old inbred mice of the Walter and Eliza Hall Institute of Medical Research were used.

Monoclonal Anti-idiotypic Antibody, 14A. A hybridoma producing anti-idiotypic antibody was prepared from an (SJL × BALB/c)F_1 mouse immunized with an A/J anti-ABA hybridoma protein, 7.1.3, which was shown to express all CRI determinants (5). The fusions were performed using polyethylene glycol, and the lines were cloned by limiting dilution. The 14A protein was affinity-purified from the 7.1.3-coupled Sepharose (5).

DTH and Transfer. Mice were injected subcutaneously for sensitization with 100 mg/kg cyclophosphamide (Endoxan, Asta, Bristol Laboratories, Crows Nest, Australia) and 2 d later painted on the clipped thorax and abdomen with 0.1 ml of a 200-mM solution of ABA diazonium in dimethylsulfoxide (5). After 5 d, they were challenged on the ears or used as donors of sensitized cells. Challenge was made by painting 10 μl of a solution of ABA diazonium or picryl chloride (0.5 mg/ml in a 50:50 vol/vol acetone/di-*n*-butyl-phthalate (British Drug Houses, Poole, U.K.). Local transfers were performed by challenging naive mice on one ear with ABA diazonium and 1 d later injecting intradermally 5×10^5 cells in 10 μl Eisen's solution (6) into both ears. After 24 h the difference between left and right ears was measured and the result was compared with the difference obtained after injection of normal cells.

Cell Preparations. Superficial lymph nodes were pressed through a stainless steel mesh into Eisen's balanced salt solution and washed. Treatment with antiserum was for 20 min on ice with washes between incubations, followed by 30 min at 37°C with rabbit complement. Anti-Thy-1.2 was an AKR anti-C3H serum diluted at 1:10. Anti-MIg was a polyvalent rabbit anti-mouse immunoglobulin (MIg) serum extensively absorbed with mouse thymus cells and used at 1:20. Anti-Ia was an A.TH anti-A.TL serum. For serum treatment, cells were suspended at 2×10^7 cells/ml. For T cell enrichment, cells were passed through nylon wool columns.

Competitive Radioimmunoassay (RIA). The RIA technique used here has been described elsewhere (5). Briefly, affinity-purified 14A Ig (2 μg/ml) or rabbit anti-CRI IgG (5 μg/ml) was

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TABLE I

14A Defines an Idiotype in A/J Mice that is ABA Specific

Sample	Dilution values or nanograms of sample required to achieve 50% inhibition of ^{125}I-7.1.3 Ig binding to either	
	Monoclonal 14A	Rabbit anti-CRI antibodies
Anti-ABA A/J serum	1:20	1:3200
Preimmune A/J serum	>1:10*	>1:10*
Anti-ABA C3H serum	>1:10*	>1:10*
ABA-BSA	10 ng	Not done
Monoclonal anti-ABA, 7.1.3	15± 3 ng	16 ± 3 ng

* Inhibitions for preimmune A/J serum tested at 1:10 were 0 ± 5% and −12 ± 4% for binding to 14A Ig and rabbit anti-CRI Ig, respectively. Corresponding values for anti-ABA C3H serum were 6 ± 3% and −14 ± 5%.

attached to microtiter wells. Dilutions of inhibitor were mixed with volume aliquots of ^{125}I-7.1.3 Ig (8×10^5 cpm/ml) before transfer of 50-μl amounts of coated wells. After an overnight incubation, wells were washed and counted in a Packard gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

Results

Monoclonal Anti-idiotype, 14A. The construction of the 14A hybridoma and the specificity of the monoclonal antibody derived from it will be described in detail elsewhere.[1] In the competitive RIA shown in Table I, low concentrations of ABA-bovine serum albumin were potent inhibitors of the reaction of 14A with the anti-ABA monoclonal reagent, 7.1.3. Pooled sera from A/J mice immune to ABA contained some antibodies reactive with 14A, whereas sera from preimmune A/J mice or ABA-immune C3H mice contained none. Hence the idiotypic determinant recognized by 14A is ABA-specific and strain-restricted and must be located at or close to the ABA-binding site. The concentration of CRI-bearing molecules in the ABA-immune A/J serum used in these assays was 200-fold higher than the concentration of 14A-reactive molecules. These must therefore represent a minor subpopulation of Ig molecules within the anti-ABA population.

Induction of DTH by 14A. A/J mice were injected in the footpads and in four subcutaneous sites with 0.4 ml of phosphate-buffered saline containing varying doses of 14A or MIg. After 5 d, their ears were challenged with ABA diazonium or picryl chloride. Significant DTH reactions to ABA, but not to picryl chloride, were found in mice receiving 2.5 or 10 μg 14A. MIg did not induce such effects (Table II).

Transfer of 14A-induced DTH by T Cells and Abrogation by 14A and Complement Treatment. A/J mice were injected with 10 μg 14A and after 5 d their lymph nodes were tested for transfer of DTH to ABA by the local transfer test. Cells from 14A-sensitized A/J mice were able to transfer ABA sensitivity (Table III). This was reduced by treatment with anti-Thy-1.2 and complement, not by anti-MIg and complement. To examine further the nature of the cells responsible for transfer, they were filtered through nylon wool, treated with anti-Ia^k serum and rabbit anti-MIg serum, and then washed and incubated with complement. After washing, the preparation was spun through fetal calf serum at 600 *g*. This produced a population of which >99% was sensitive to anti-Thy-1.2 serum and complement. This enriched T cell population

[1] Morahan, G., and I. D. Walker. Immunochemical properties of a monoclonal antiazobenzenearsonate idiotype antibody. Manuscript in preparation.

TABLE II

Induction of DTH to ABA in A/J Mice by the Monoclonal Anti-Idiotype 14A

Sensitizing injection	Challenge at 5 d	Ear increment (10^{-2} mm) (SD)*
None	ABA	2.3 (0.7)
2.5 μg 14A monoclonal	ABA	4.7 (1.6)
10 μg 14A monoclonal	ABA	6.1 (1.1)
40 μg 14A monoclonal	ABA	3.8 (3.3)
10 μg 14A monoclonal	Picryl chloride	2.1 (0.6)
None	Picryl chloride	2.3 (0.1)
10 μg MIg	ABA	2.6 (1.1)

* Mice injected with 2.5 and 10 μg 14A had significant responses compared with nonsensitized controls ($P < 0.02$, $P < 0.01$, respectively). Mean (SD) five mice per group.

TABLE III

Transfer of DTH to ABA from A/J Mice Sensitized with Monoclonal Anti-Idiotype 14A

Group	A/J lymph node cells used for transfer (5×10^5)	Incubations		Ear increment (10^{-2} mm) after ABA challenge (SD)*
		On ice	At 37°C	
1a	Normal	—	—	2.2 (3.1)
1b	14A sensitized	—	c′	10.9 (3.5)
1c	14A sensitized	Anti-Thy-1.2	c′	1.7 (1.6)
1d	14A sensitized	Anti-MIg	c′	15.4 (3.3)
1e	14A sensitized	14A, anti-MIg‡	c′	4.8 (1.3)
2a	Normal	—	—	2.4 (1.4)
2b	14A sensitized, nylon wool filtered	—	—	6.3 (1.2)
2c	14A sensitized, nylon wool filtered	Anti-Ia^k, anti-MIg§; MIg, anti-MIg‖	c′	5.2 (0.7)
2d	14A sensitized, nylon wool filtered	Anti-Ia^k, anti-MIg§; 14A, anti-MIg‖	c′	2.0 (1.3)
3a	Normal	—	—	0.7 (1.8)
3b	ABA sensitized	Anti-MIg	c′	5.7 (1.8)
3c	ABA sensitized	14A, anti-MIg‡	c′	5.5 (2.8)

* Mean (SD) of groups of five mice. *P* values: 1b, 1c < 0.01; 1d, 1e < 0.01; 2a, 2b < 0.01; 2a, 2c < 0.01; 2c, 2d < 0.01.
‡ Cells washed between incubations.
§ Cells were incubated with anti-Ia and anti-MIg, then washed and incubated with complement (c′).
‖ Cells were incubated with MIg or 14A, washed, incubated with anti-MIg, washed, and then incubated with complement (c′).

could transfer DTH to ABA. To determine whether these cells carried the idiotypic determinant detected by 14A, they were incubated with 10 μg/ml 14A or MIg, washed and incubated with rabbit anti-MIg serum, and finally washed and treated with rabbit complement. The transfer of DTH was abrogated by treatment with 14A, anti-MIg and complement, not by treatment with MIg, anti-MIg, and complement.

14A-induced DTH is Major Histocompatibility Complex (MHC)-restricted. Cells from mice treated with 14A to induce DTH were transferred to naive recipients of various genotypes to test for MHC restriction. Identity at the D-end was not sufficient to allow transfer from A/J to BALB/c mice (group 1d), even though BALB/c could support transfer (group 2d). The failure to transfer was not the result of some allogeneic reaction because transfer was possible in various parental-F_1 combinations (Table IV).

Discussion

The 14A monoclonal anti-idiotype was prepared by cell fusion techniques from mice injected with the hybridoma protein, 7.1.3, which contains the entire ABA CRI of A/J mice (5). The specificity of 14A was demonstrated by its reaction with 7.1.3

TABLE IV

MHC Restriction of the Transfer of ABA-specific DTH Induced by the Monoclonal Anti-Idiotype Reagent 14A

Group	Lymph node cells used for transfer (5×10^{-5})	Recipient strain	MHC of recipient*	Ear increment (10^{-2} mm) after ABA challenge (SD)‡
1a	Normal A/J	A/J	kkkkkddd	1.8 (0.9)
1b	14A-sensitized A/J	A/J	kkkkkddd	6.5 (1.8)
1c	Normal A/J	BALB/c	dddddddd	2.2 (1.6)
1d	14A-sensitized A/J	BALB/c	dddddddd	1.5 (1.2)
1e	Normal A/J	(A/J × BALB/c)F_1	kkkkkddd × dddddddd	1.5 (0.9)
1f	14A-sensitized A/J	(A/J × BALB/c)F_1	kkkkkddd × dddddddd	6.3 (2.0)
2a	Normal (A/J × BALB/c)F_1	(CBA × BALB/c)F_1	kkkkkkkk × dddddddd	1.3 (1.1)
2b	14A-sensitized (A/J × BALB/c)F_1	(CBA × BALB/c)F_1	kkkkkkkk × dddddddd	5.9 (1.4)
2c	Normal (A/J × BALB/c)F_1	BALB/c	dddddddd	1.1 (1.2)
2d	14A-sensitized (A/J × BALB/c)F_1	BALB/c	dddddddd	6.3 (1.0)

* MHC regions shown are: K, I-A, I-B, I-J, I-E, I-C, S, and D.

‡ Mean (SD) of five mice per group. *P* values: 1a, 1b < 0.001; 1e, 1f < 0.01; 2a, 2b < 0.001; 2c, 2d < 0.001.

and its failure to react with nonimmune A/J serum and anti-ABA serum from C3H mice. A high concentration of A/J anti-ABA serum was, however, required to inhibit the reaction between 14A and 7.1.3, which suggests that the determinant recognized by 14A is expressed only on a minor population of CRI^+ antibodies.

DTH to ABA, but not to picryl chloride, could be induced by injecting A/J mice subcutaneously with 14A. The sensitivity could be transferred to naive mice by T cells because anti-Thy-1.2 and complement abolished transfer and because T cells highly enriched by a combination of nylon wool filtration and sequential treatment with anti/Ia, anti-MIg, and complement could transfer ABA sensitivity. Treatment of these enriched cells with 14A in combination with anti-Ig and complement considerably reduced transfer. The requirement for anti-Ig could reflect a low density of idiotype on the cell or the poor complement-fixing ability of 14A (an IgG_1 protein).[1] The abrogation of transfer by highly enriched T cells after treatment with the anti-idiotype reagent does, however, imply a direct interaction between the T cell and the anti-idiotype. Whether this interaction per se is sufficient to activate all the T cells involved in the sensitivity or whether it is followed by a series of idiotype-anti-idiotype-driven interactions is not known.

The anti-idiotype-induced DTH was MHC restricted. If the assumption is made that anti-idiotype activates T cells not after macrophage processing, but directly via an anti-ABA receptor, the results could be interpreted to imply that MHC restriction is preexisting and not imposed only after antigenic stimulation.

Anti-idiotype antibodies of different Ig classes have been reported as having differential abilities to induce helper or suppressor effects (1). Monoclonal anti-idiotypes differing in isotype seem to provide ideal reagents to study this phenomenon. Their ability to activate T cells which bear the corresponding idiotype, as shown in this study, also makes them powerful tools to investigate the structure of the T cell receptor on corresponding lines of T cells or hybridomas.

Summary

Azobenzenearsonate (ABA)-specific sensitivity was induced in A/J mice by injecting a monoclonal anti-idiotype reagent, 14A, directed against a determinant present on

a minor subpopulation of immunoglobulin molecules within the anti-ABA antibodies of A/J mice. Sensitivity was transferrable by purified T cells and this was abrogated by treating the cells with 14A, rabbit anti-mouse immunoglobulin and complement, not by treatment with only the last two reagents. The transfer was restricted by the K-end of the major histocompatibility complex.

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