PURIFICATION OF FUNCTIONAL, DETERMINANT-SPECIFIC, IDIOTYPE-BEARING MURINE T CELLS*

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Even though it has been relatively easy to demonstrate the immunogenicity of azobenzenearsonate compounds in the guinea pig (1–5) and the rat (6), the mouse has distinct advantages in terms of ease of T-cell identification and the characterization of idiotypic markers associated with anti-azobenzenearsonate (ABA)[1] specificity. A major cross-reactive idiotype (CRI) found on 20–70% of the anti-ABA antibodies produced by individual strain A/J mice has been extensively studied by Nisonoff et al. (7). We have recently shown that monofunctional ABA-tyrosine (RAT) induces T-cell responses in A/J mice, determined by the appearance of antigen-binding cells and the development of delayed hypersensitivity (8). Anti-ABA antibody could not be detected in animals immunized with the monofunctional compound, but a major fraction of ABA-binding T cells expressed the CRI, as determined by the blocking activity of anti-CRI antibody. Furthermore, A/J mice make T-dependent antibody responses to bifunctional conjugates of RAT and of ABA-histidine (8). These conjugates evoke a primary anti-dinitrophenyl (DNP) response peaking at 7 days with $\cong 3 \times 10^3$ IgM plaque-forming cells (PFC)/spleen. This IgM response occurs without an apparent shift to IgG production, and is accompanied by only weak immunological memory. Thus, whereas the response of A/J mice to mono- and bifunctional ABA antigens serves to demonstrate the existence of T-cell activity to this determinant and the expression of the CRI on a fraction of the T cells, their magnitude is insufficient to permit the isolation of numbers of T cells required for characterization of the antigen receptor.

Recently, several laboratories have used hapten-coupled autologous immunoglobulins to generate substantial hapten-specific helper (9) and suppressor (10) activity in mice. We now describe this approach to generate a sizable pool of functional ABA-specific T cells in A/J mice, many of which express the CRI, as well as the subsequent purification and characterization of the cells.

Materials and Methods

Mice. Female A/J mice 6–8 wk of age were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Antigens and Immunization. Giant keyhole limpet hemocyanin (KLH) was purchased from

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[1] Abbreviations used in this paper: ABA, azobenzenearsonate; BGG, bovine gamma globulin; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; CRI, cross-reactive idiotype; DNP, dinitrophenyl; FCS, fetal calf serum, HBSS, Hanks' balanced salt solution; IFA, incomplete Freund's adjuvant; KLH, keyhole limpet hemocyanin; MSA, mouse serum albumin; PFC, plaque-forming cells; RAM Ig, rabbit anti-mouse Ig; RAT, L-tyrosine-*p*-azobenzenearsonate; TNP, trinitrophenyl.

Calbiochem, San Diego, Calif. Mouse serum albumin (MSA) and bovine serum albumin (BSA) were obtained from Miles Laboratories Inc., Elkhart, Ind. Mouse IgG was isolated from either adjuvant-induced ascites fluid in A/J mice or from ascites fluid induced by MOPC-21 (γ_1, κ) myeloma cells. Gamma globulins were precipitated with 18% sodium sulfate and purified further by ion exchange chromatography on DEAE-cellulose. The IgG fraction was eluted using a linear phosphate gradient (0.005 M starting buffer, 0.13 M limit buffer) at pH 8.0. Although the IgG so obtained was immunoelectrophoretically pure, analysis by sodium dodecylsulfate-polyacrylamide gel electrophoresis (11) revealed a number of minor contaminants of varying molecular size; therefore, the IgG was purified further by chromatography on Bio-Gel A1.5M (borate-buffered saline, pH 8.0).

ABA-protein conjugates were prepared according to Tabachnick and Sobotka (12). Since it has not been possible to dissect the relative contributions of ABA-tyrosine and ABA-histidine to the absorption spectra of ABA-proteins using synthetic model compounds (D. E. Nitecki, unpublished observations), no attempt was made to determine the degree of azo-coupling. Trinitrophenyl $(TNP)_{180}$-KLH conjugates were prepared according to Rittenberg and Amkraut (13), and doubly conjugated proteins were synthesized according to Amkraut et al. (14). RAT was synthesized as described previously (2).

For immunization, the proteins were dissolved in phosphate-buffered saline at 2 mg/ml^{-1} and emulsified with an equal volume of complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, Mich.). Typically, each A/J mouse received a single injection of 100 μg of antigen intraperitoneally in 0.1 ml CFA, except when indicated otherwise.

Antisera. Rabbit anti-mouse Ig (RAM Ig) was prepared using normal A/J immunoglobulin, obtained from serum by sequential precipitation with 18 and 12% sodium sulfate, emulsified in CFA (final concentration 1 mg/ml^{-1}) to immunize New Zealand white rabbits (Simonsen Laboratories, Gilroy, Calif.). The rabbits were exsanguinated 2 wk after three biweekly subcutaneous injections of 1 mg of antigen. The resulting antibody was purified by affinity chromatography using normal A/J Ig conjugated to Sepharose 4B, prepared according to Hudson and Hay (15). The specific RAM Ig was eluted with 3 M ammonium thiocyanate, pH 8.0, and dialyzed against phosphate-buffered saline. The antibody was brought to 1 mg/ml^{-1}, sterilized by filtration, and stored at 4°C. This preparation could be used at least 12 times to coat Petri plates for removal of B cells from spleen cell suspensions (see below).

Anti-Thy-1.2 ascites fluid was purchased from Litton Bionetics, Kensington, Md. Rabbit anti-mouse brain was prepared according to Golub (16), using tissue from CBA mice for all immunizations.

A/J anti-ABA ascites was prepared by repeated inoculation of 500 μg of ABA-KLH in CFA as described by Tung et al. (17). Specific anti-ABA antibody was purified from the ascites by affinity chromatography on Sepharose 4B conjugated with ABA-bovine gamma globulin (BGG) (15). The antibody was eluted with 3 M ammonium thiocyanate, pH 8.0, dialyzed against water, and lyophilized. For preparing anti-CRI, the A/J anti-ABA antibody was emulsified in CFA, and 1 mg was injected subcutaneously into each of three New Zealand white rabbits. 1 mo later, the rabbits were given a booster injection and were then bled periodically. A high-titered antiserum was obtained and rendered idiotype-specific by absorption with normal A/J immunoglobulin conjugated to Sepharose 4B (15). Using a tube binding assay for anti-CRI activity (18), the binding of anti-ABA antibody by this antiserum was inhibited 50% by 50 ng/ml^{-1} of unlabeled antibody, but not at all by 200 μg/ml^{-1} of normal A/J IgG.

Fluoresceinated rabbit anti-DNP $F(ab')_2$ fragments were prepared by standard procedures as outlined by Hudson and Hay (15).

A.TH anti-A.TL antiserum (anti-Ia^k) was purchased from Searle Diagnostics, High Wycombe, England.

Cell Fractionation. Cell suspensions were prepared by gently teasing spleen in Hanks' balanced salt solution (HBSS) containing 10% fetal calf serum (FCS). Large aggregates were allowed to settle over a gradient of FCS at 4°C, after which fine material was removed by centrifugation over a fresh FCS gradient. B cells were removed according to Mage et al. (19) by allowing the spleen cell suspensions to settle onto plastic Petri plates precoated with affinity-purified RAM Ig. This procedure routinely yielded T-cell preparations that were >95% pure, as judged by anti-Thy-1 cytotoxicity and staining with fluorescent anti-Ig. Alternatively, in

some experiments B cells were depleted using nylon wool columns exactly as described by Julius et al. (20). Antigen-binding T cells were purified using the procedure of Taniguchi and Miller (21), in which splenic T cells were allowed to settle onto plates previously coated by overnight incubation with ABA-mouse IgG, ABA-MSA, or ABA-BGG at 1 mg/ml^{-1}. After settling for 1 hr at room temperature, the nonadherent T cells were decanted and the plates were gently washed twice. To elute the bound cells, the plates were placed on ice for 30 min, after which the bound cells were easily removed by gentle pipetting. The recovered adherent cells were washed twice before analysis.

Analysis of Antigen-Binding Cells. To assay for antigen-binding cells, lymphocytes were brought to ≅ 10^7 cells/ml^{-1} in HBSS with 10% FCS (HBSS/FCS), and mixed with an equal volume of doubly conjugated (DNP, ABA)-BSA (1 mg/ml^{-1}) in HBSS/FCS with 1 mg/ml^{-1} sodium azide. After incubating for 20 min on ice, the cells were spun through a gradient of FCS and resuspended in HBSS/FCS containing 50 μg ml^{-1} fluorescein-conjugated F(ab')$_2$ fragments of rabbit anti-DNP antibody. The cells were incubated for an additional 20 min on ice, spun through FCS, resuspended in HBSS/FCS, and scored for fluorescence using a Zeiss fluorescent microscope (Carl Zeiss, Inc., New York).

To investigate the cellular expression of CRI, ABA-specific T cells purified on antigen-coated plates were suspended at 10^7 cells/ml^{-1} in HBSS containing 50 μg/ml^{-1} anti-CRI and 500 μg/ml^{-1} sodium azide. After incubating on ice for 20 min, the cells were washed three times and incubated with 10 μg ml^{-1} of fluorescein-conjugated staphylococcal protein-A (Pharmacia Inc., Piscataway, N. J.) in HBSS/azide for 20 min, washed twice, and examined for fluorescence. Alternatively, the capacity of anti-CRI to inhibit antigen-binding by T cells was assessed by incubation with the antiserum, as above, before the assay for antigen binding.

Cell Surface Markers. B cells were detected using fluorescein-conjugated RAM Ig (Antibodies Inc., Davis, Calif.). This procedure was identical to that described above for anti-idiotype staining except that 50 μg/ml^{-1} fluoresceinated RAM Ig was substituted for anti-CRI and protein-A. Ia^k antigens and Thy-1.2 antigens were detected using a cytotoxicity assay described previously (22).

Cell Transfers. 2 days before cell transfer, recipient mice received 500 μg of gentamycin i.p., and they were placed on chlorinated acid water. On the day of transfer, the mice received 650 rads whole body x-irradiation. 10 min before cell transfer, recipient mice received 10 U of heparin i.p. in HBSS. Cells were transferred in a total volume of 0.5 ml in HBSS containing the boosting antigen.

Plaque Assays. 7 days after cell transfer, anti-TNP PFC were detected using TNP-sheep erythrocytes (23) and a slide modification of the Jerne plaque assay (24). BSA-specific plaques were detected using the carbodiimide coupling procedure described by Golub et al. (25) to prepare indicator erythrocytes.

Results

ABA-Specific Helper and Suppressor Activity. In early experiments aimed at generating a substantial pool of ABA-specific T cells, A/J mice were given four weekly injections of 100 μg of ABA-MOPC-21 conjugates. The first dose was incorporated in CFA, the second in incomplete Freund's adjuvant (IFA), and the last two in saline. The mice were then rested for at least 1 mo. Other A/J mice were primed with 100 μg of BSA in CFA to serve as a source of B cells for adoptive transfer experiments. The spleens from 10 A/J mice primed with ABA-IgG were pooled, a T-enriched fraction was obtained by nylon wool passage, and the T cells were assayed for ABA-specific help and suppression in adoptive anti-BSA responses (Table I). Transferring 2 × 10^7 BSA-primed spleen cells into irradiated mice and challenging with ABA-BSA gave an anti-BSA response of 16,200 ± 1,707 IgG PFC/spleen at 7 days (group I). In contrast, when 1 × 10^7 ABA-IgG-primed T cells were cotransferred with BSA-primed spleen cells, the anti-BSA response was dramatically suppressed (793 ± 542 IgG PFC/spleen [group II]). On the other hand, the response of BSA-primed B cells (spleen cells

TABLE I

ABA-Specific Helper and Suppressor Activity

Group*	ABA-Primed T Cells‡	BSA-Primed B Cells§	BSA-Primed spleen cells	Anti-BSA IgG PFC/Spleen
I	—	—	2×10^7	16,200 ± 1,707
II	1×10^7	—	2×10^7	793 ± 542
III	1×10^7	5×10^6	—	7,380 ± 1,002
IV	—	5×10^6	—	246 ± 410

* All mice were irradiated with 650 rads, challenged with 100 μg ABA-BSA i.p. in saline after cell transfer, and assayed for anti-BSA 7 days later.

‡ Nylon wool-passed spleen cells from mice primed with ABA-IgG_1 (>99% Thy-1.2-positive).

§ Anti-Thy-1.2-plus-complement-treated spleen cells from mice primed with BSA.

treated with anti-Thy-1.2 serum plus complement) was markedly helped by ABA-primed T cells (group III versus group IV), suggesting the requirement for interaction between these T cells and cells removed by anti-Thy-1.2 treatment of spleen cells in the generation of suppression. The specificity of the ABA-primed T cells was shown by their inability to modulate the anti-ABA responses of spleen cells or B cells when BSA rather than ABA-BSA was used as the immunogen in irradiated, reconstituted animals, and by the inability of T cells from normal A/J mice to exert a significant regulatory effect on the response of BSA-primed cells to ABA-BSA. The results indicate that significant coexisting epitope-specific helper and suppressor activities can be generated in A/J mice by priming with ABA-mouse IgG.

The above findings for an anti-protein PFC response were confirmed for an anti-hapten response, using a protocol with which suppressor activity induced by a monofunctional ABA antigen (RAT) was also studied. Here, unfractionated spleen cells from mice primed 2 wk earlier with ABA conjugates were co-transferred with spleen cells from mice primed at least 6 wk earlier with 100 μg of TNP-KLH in CFA. This protocol for priming ABA-specific T cells was adopted because it reportedly optimizes the expression of suppressor activity (10). The recipients were boosted with 100 μg of doubly conjugated (TNP,ABA)-KLH and the anti-TNP IgG PFC responses were assayed 7 days later (Table II). ABA-specific suppression was readily induced by ABA-IgG in CFA (group II) or RAT in IFA (group IV), whereas RAT in CFA (group III) was less effective and more variable for suppressor induction. The results strengthen the case for the existence of ABA-specific suppressor T cells, since their induction by monofunctional ABA compounds vitiates the argument that suppressor cells might be directed against altered determinants of protein carriers (ABA-IgG and ABA-BSA).

Purification of ABA-Specific T Cells. The demonstration of ABA-specific T-cell functional activity in A/J mice prompted attempts to purify such cells. Whereas helper cells are generally enriched on monolayers of antigen-pulsed macrophages (26), suppressor cells have been enriched on antigen-coated Petri plates (21) or antigen-derivatized Sephadex columns (10). Since suppression appeared to be dominant in the response of A/J mice to ABA-IgG, the technically simple procedure described by Taniguchi and Miller (21) was adopted to enrich ABA-specific T cells. Spleen cells from mice sensitized with ABA-IgG were initially depleted of B cells by incubation on plates coated with affinity-purified RAM Ig antibody. We have consistently found that ≅ 30% of the spleen cell population is nonadherent to anti-Ig plates; this fraction

TABLE II

Induction of ABA-Specific Suppression of Anti-TNP Responses by ABA-IgG$_1$ and Monofunctional ABA-Conjugates

Group*	Responding cells	Suppressor cells	Anti-TNP IgG PFC/spleen	Suppression
				%
I	2.5×10^7 TK‡	2.5×10^7 normal spleen	72,700 ± 11,464	0
II	2.5×10^7 TK	2.5×10^7 ABA-IgG$_1$/CFA§	23,520 ± 1,099	68
III	2.5×10^7 TK	2.5×10^7 RAT/CFA ‖	44,760 ± 18,644	38
IV	2.5×10^7 TK	2.5×10^7 RAT/IFA¶	34,260 ± 8,763	53

* Irradiated recipient mice challenged with doubly conjugated (TNP, ABA)-KLH.

‡ TK, spleen cells from A/J mice primed 6 wk earlier with 100 μg of TNP-KLH/CFA.

§ Spleen cells from mice primed 2 wk earlier with 100 μg ABA-IgG$_1$/CFA.This protocol was found to be optimal for induction of suppression in pilot experiments.

‖ Spleen cells from mice primed 2 wk earlier with 100 μg RAT/CFA.

¶ Spleen cells from mice primed 2 wk earlier with 100 μg RAT/IFA.

is >95% Thy-1-positive and <5% Ig-positive on the basis of cytotoxicity and fluorescent staining, respectively. We consider this method of enriching T cells superior to nonspecific techniques such as nylon wool passage, which may remove a significant fraction of T cells along with B cells (27).

The splenic T cells were incubated for 1 h at room temperature on polystyrene culture plates coated with ABA-protein conjugates. After incubation, the nonadherent cells were removed and the plates were transferred to an ice bath. The adherent cells are released within 15–30 min on ice and can be removed by gentle pipetting. Essentially all of the T cells exposed to the plates can be accounted for in the recovered adherent and nonadherent fractions. Approximately 6% of the T cells (2% of unfractionated spleen cells) bind to the plates and ≅ 90% of this adherent population was found to bind antigen immediately after recovery (Table III). In contrast, no ABA-binding cells were found in the nonadherent fraction. T cells from unimmunized mice did not bind to ABA plates in detectable numbers.

A comparison of the adherent and nonadherent T cells recovered from ABA-IgG-coated plates is summarized in Table III. Both the antigen-adherent and nonadherent fractions were >95% T cells as judged by lysis with anti-Thy-1 serum plus complement. Surprisingly, virtually all the adherent cells stained with fluorescent anti-mouse Ig antibody. However, the staining of these cells was very dull and patchy as opposed to the vivid, uniform fluorescence seen in stained spleen cells, B cell preparations, or the 5% of nonadherent cells which stained with the anti-Ig reagent. Therefore, it seemed probable that the Ig on adherent cells was acquired during purification on ABA-Ig-coated plates. This premise was confirmed by the absence of Ig-positive T cells recovered from plates coated with ABA-MSA. This latter protein was not used in early experiments, but it and ABA-BGG have been found to provide the same binding efficiency as plates coated with ABA-IgG. The mean binding in six experiments using isologous IgG, MSA, and BGG conjugates was 1.95 ± 0.95% of the total spleen cell population.

Another distinguishing feature of the adherent and nonadherent T-cell populations was the expression of Ia antigen(s) on the majority of adherent cells but on only a very minor fraction of nonadherent cells, as judged by cytotoxicity with an A·TH

TABLE III

Characterization of Adherent and Nonadherent ABA-Ig Immune T Cells Incubated on ABA-IgG$_1$-Coated Plates

T-Cell type	Fraction of total	Thy-1	Ig	Ia*	Antigen binding‡	CRI§
		%	%	%	%	%
Adherent	0.06	95	100 ‖	78	91	54 ± 4
Nonadherent	0.94	95	5¶	2	0	0

* Cytotoxicity of A·TH anti-A·TL serum plus complement.

‡ Cells were incubated with (ABA, DNP)-BSA followed by reaction with fluoresceinated (Fab')$_2$ fragments of anti-DNP antibody.

§ Cells were reacted with rabbit anti-CRI serum, washed, and reacted with fluorescent protein A.

‖ Very weak, patchy fluorescence.

¶ Strong, ring fluorescence.

anti-A·TL serum plus complement. Since this antiserum recognizes determinants coded by the entire known I region, the Ia subtype of the cells was not identified, but it seems plausible that many of the cells might express I-J markers since I-J is the predominant Ia locus associated with suppressor activity.

Perhaps the most exciting finding was the expression of the CRI by ABA-binding T cells recovered from ABA-MSA-coated plates. The expression of the idiotype was assayed two ways. In one, cells were incubated with anti-CRI before testing for antigen binding. This inhibition assay decreased the number of antigen-binding cells by an average of 44%, suggesting the presence of an idiotypic marker in the receptor binding site of the inhibited population. The second procedure was a direct assay in which cells were reacted with anti-CRI, washed, and incubated with fluorescent protein A to reveal anti-CRI-binding T cells. The rabbit anti-CRI serum was absorbed with normal T cells and showed no nonspecific staining. Using this assay, 54 ± 4% of the adherent cells stained for CRI, whereas the nonadherent T cells showed no reaction. Furthermore, adherent T cells that were not exposed to anti-CRI did not bind fluorescent protein A. Thus, the findings establish the expression of the CRI on a major fraction of ABA-binding T cells, but they do not ascertain the biosynthetic origin of the idiotype-bearing molecule.

The biological function of the T cells binding to ABA-coated plates was investigated using a standard cell transfer protocol (Table IV). Transferring equal numbers (2.5×10^7) of TNP-KLH-primed spleen cells and ABA-IgG-primed spleen cells reduced the IgG anti-TNP PFC response of irradiated recipients to doubly conjugated (TNP,ABA)-KLH by 53%. Smaller numbers of the suppressor population gave <50% reduction of the response. T cells that did not adhere to ABA-Ig-coated plates were only marginally capable of suppressing the anti-TNP response (group III). In marked contrast, adherent T cells were highly suppressive at 1/25 the number of unfractionated spleen cells required for significant suppression (compare groups II and IV). The results establish a highly significant enrichment of functional, determinant-specific suppressor cells in preparations of splenic T cells which adhere to ABA-coated plates.

Discussion

The chemical nature of the T-cell receptor for antigen and its relationship to conventional immunoglobulins is one of the central unresolved issues in immunology.

TABLE IV

Suppressor Function of T Cells Binding to ABA-Coated Plates

Group*	Responding cells	Suppressor cells	Log PFC/spleen	Suppression
				%
I	2.5×10^7 TK spleen‡	2.5×10^7 normal spleen	4.99 ± 0.02 (97, 723)§	—
II	2.5×10^7 TK spleen	2.5×10^7 ABA-IgG$_1$ spleen ‖	4.66 ± 0.12 (45,708)	53
III	2.5×10^7 TK spleen	2.5×10^7 ABA-Ig nonadherent¶	4.89 ± 0.04 (77,624)	20
IV	2.5×10^7 TK spleen	1.25×10^6 ABA-Ig adherent¶	4.46 ± 0.38 (28,840)	70

* All irradiated recipient mice were boosted with 100 μg (TNP, ABA)-KLH in CFA after cell transfers.

‡ TK, spleen cells from A/J mice primed 6 wk earlier with 100 μg of TNP-KLH/CFA.

§ Numbers in parenthesis represent mean IgG PFC/spleen.

‖ Spleen cells from mice primed 2 wk earlier with 100 μg ABA-IgG$_1$-CFA.

¶ T cells from ABA-IgG$_1$-primed mice fractionated by adherence to ABA-Ig-coated plates.

There is persuasive evidence that specific antibodies share idiotypic markers with otherwise dissimilar antigen receptors on T cells (28, 29). This evidence argues for some degree of binding-site similarity with extensive framework dissimilarity between the two types of antigen-recognizing molecules. A particularly attractive idiotypic model with which to explore the structural relationships between antibodies and T-cell receptors is the cross-reactive idiotype found on 20–70% of anti-ABA antibodies produced by individual A/J mice (7). Not only is it a major idiotypic species in this strain of mice, but it is associated with a well-defined antibody specificity. Furthermore, the structural characterization of CRI-bearing antibody molecules is already well under way (30, 31).

Our interest in the A/J CRI was catalyzed by our experience with T-cell specificity directed against the ABA epitope in guinea pigs (1–3). When we turned our attention to mice, we established that monofunctional as well as multifunctional ABA compounds could induce the appearance of ABA-binding T cells and ABA-specific helper activity in A/J mice (8). Furthermore, many of the antigen-binding T cells reacted with anti-CRI antibody. The evidence that T cells were not expressing a passively acquired antibody was based on the appearance of such cells after immunization with monofunctional ABA compounds which do not elicit detectable PFC responses, and on reappearance of the receptor after tryptic removal (8). These findings heightened our interest, but a major deterrent to further progress was posed by the relatively weak responses obtained; the proportion of T cells from immune animals that bound ABA ranged from 0.01 to 0.1%. Therefore, we concentrated on mechanisms for amplifying the ABA-specific T-cell response antecedent to the purification of such cells.

The realization of these objectives was immeasurably abetted by two recent observations. The first was that hapten conjugates of autologous immunoglobulin were potent inducers of suppressor activity (10). The second was that suppressor cells, in contrast to helpers, bind to antigen-coated matrices and can be recovered by a simple temperature-dependent elution process. Accordingly, we adopted the ligand-coated polystyrene culture dish technique of Taniguchi and Miller (21), which, in turn, was based on a method described by Mage et al. (19), to purify antigen-binding T cells generated by immunization with ABA conjugates of the mouse IgG$_1$ myeloma protein, MOPC-21.

The effectiveness of this protocol exceeded our expectations. A single injection of 0.1 mg of ABA-IgG led 2 wk later to the appearance of a population of ABA-binding T cells which comprised ≅ 2% of the total spleen cell population. ABA-specific T cells

raised by ABA-IgG could manifest help or suppression depending on the cell transfer protocol (Table I). It is not known whether the antigen-binding assay detected helpers as well as suppressors, nor can we conclude that the T cells active in suppression in the ABA-IgG-primed population were effector suppressors. Based on the evidence for interaction between these cells and another population of T cells for optimal generation of suppression (Table I), they may function as precursors of suppressor cells or as helpers in the differentiation of effector suppressor cells.

Be that as it may, the fact remains that ABA-IgG generated a pool of ABA-binding T cells at least an order of magnitude larger than the numbers generated by monofunctional ABA compounds or by ABA-KLH conjugates (8). These cells were very efficiently purified by first removing Ig-positive cells by adherence to anti-Ig-coated plates, followed by adherence of the ABA-specific T cells to and subsequent elution from ABA-coated plates. The antigen-adherent and nonadherent cells accounted for virtually 100% of the T cells in the original spleen cell population, and $\cong$ 90% of the adherent cells bound antigen immediately after recovery from the plates (Table III). 78% of the adherent cells reacted with an I-region antiserum (A·TH-anti-A·TL), whereas only 2% of the nonadherent did so. Although the particular I-region marker expressed by the adherent cells could not be identified with the reagents available, it is likely to be the Ia-4 determinant defined by the I-J subregion associated with suppressor activity, since suppressor cells are known to be selectively retained by antigen-coated surfaces and the suppressor activity of the purified cells was increased at least 25-fold (Table IV).

The expression of the CRI on about half of the ABA-binding T cells (or 1–2 $\times$ 10^6 cells/spleen) (Table III) makes feasible attempts to isolate the idiotype-bearing T-cell receptor, perhaps not directly, but possibly by producing T-cell hybridomas which express the idiotype. Although the biosynthetic origin of the CRI on T cells after ABA-IgG immunization was not established, the CRI does appear on T cells after immunization with monofunctional ABA compounds which do not induce detectable anti-ABA PFC responses, and it has reappeared after removal by trypsin (8). Hence, it is likely to be a product of the T cell itself.

Summary

Strain A/J mice immunized with azobenzenearsonate (ABA)-mouse IgG conjugates develop suppression for anti-trinitrophenyl(TNP) responses to doubly conjugated (ABA,TNP) proteins. This suppression is specific for the ABA epitope and is mediated by T cells in cell transfer experiments. ABA-binding T cells from suppressed animals were purified by a two-stage procedure in which B cells were removed from spleen cell populations by adherence to plastic surfaces coated with anti-mouse Ig antibody, followed by binding the nonadherent population (>95% Thy-1-positive) to surfaces coated with ABA-protein conjugates. Approximately 90% of the cells recovered by temperature-dependent elution from the ABA plates ($\cong$ 2% of the spleen cells) bound antigen immediately afterward, and up to 50% of the cells bound anti-cross-reactive idiotype antibody. On the other hand, the nonadherent T-cell population was completely negative in the antigen-binding and idiotype assays. Another distinguishing feature of the two T-cell populations was that 78% of the adherent cells, but only 2% of the nonadherent cells, were Ia positive, although the specific I-region marker(s) expressed on the cells was not identified. The biological function of the antigen-

binding T cells was investigated using a standard cell transfer protocol. Suppressor cells were enriched in the adherent population by a factor of at least 25, establishing that functional, epitope-specific, idiotype-bearing T cells can be significantly purified by this procedure.

Note Added in Proof. We have recently isolated two types of ABA-binding molecules biosynthetically labeled with ^{35}S-methionine from NP-40 lysates of purified antigen-specific T cells. The molecules were purified by adsorption onto an ABA-Sepharose immunoadsorbent followed by elution with 9 M urea. Autoradiograms of SDS-PAGE of the eluates revealed components with mol wt of approximately 60,000 and 33,000 daltons. These molecules were not present in eluates from a bovine IgG-Sepharose control immunoadsorbent and thus represent specific ABA-binding products synthesized by T cells.

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