BINDING OF PURIFIED, SOLUBLE MAJOR HISTOCOMPATIBILITY COMPLEX POLYPEPTIDE CHAINS ONTO ISOLATED T-CELL RECEPTORS

I. Reactivity against Allo- and Self-Determinants*

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The spectrum of immune reactivity of T lymphocytes seems to be skewed toward anti-major histocompatibility complex (MHC)[1] reactivity when compared to that of B cells. T lymphocytes are thus known to display striking reactivity toward the major histocompatibility-locus alloantigens as measured in mixed lymphocyte cultures (MLC) (1), graft-versus-host reaction (2–4) or by using anti-idiotype antibodies when to determine the actual numbers of T cells participating in such reactions (5). No similar preoccupation has been noted for B lymphocytes (5). It is, in this context, quite crucial to relate the high number of alloreactive T lymphocytes with the recently described self-MHC reactivity displayed by helper or killer T lymphocytes (6–8). Here, both helper and killer cells display clonally distributed receptors for self-MHC molecules (helper cells for self-Ia) (6) and killer lymphocytes for certain types of beta$_2$ microglobulin-containing MHC structures (7, 8). A selection for anti-self MHC reactivity occurs by unknown mechanisms via the MHC determinants of the thymic epithelium (9, 10).

The above findings have led to several theories with regard to T-cell specificity. According to one major group of theories, the T cell must be equipped with two sets of receptors, with one set occupied with self-MHC reactivity and, the other set would have specificity for conventional antigens. According to the second group of theories, the T-cell receptors present on a single lymphocyte would only be of one kind, but would be a rather complicated molecule with the ability to see pieces of conventional antigen in actual physical contact with MHC molecules of relevant type on the stimulator/target cell (8). The problem of whether anti-allo-MHC-reactive T cells also belong to the anti-self-MHC-reactive cells or constitute their own unique set of

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[1] *Abbreviations used in this paper:* CNBr, cyanogen bromide; D-PBS, phosphate-buffered saline; EHAA, Eagle's high amino acid; FCS, fetal calf serum; LD, lymphocyte-defined; LPS, lipopolysaccharide; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; NP40, Nonidet P-40; SD, serum-defined; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel.

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cells is still a matter of debate. Many workers have however described that allo-activated T lymphocytes in cytolytic systems may also express significant, although weak, reactivity toward modified syngeneic targets or opposite (11–13). Similarly, it has been possible to select specific T-cell lines with reactivity both toward allo-MHC and toward minor histocompatibility antigens in the context of self-MHC determinants (12). These latter data do not provide, however, any information as to the possibility of several distinct sets of receptor molecules on the reactive T lymphocytes.

In this study we have tried to make some conclusive experiments in relation to the above findings. We considered that this should be possible via studies of the fine antigen-binding ability of purified idiotype-positive molecules derived from T lymphocytes. Our approach involves the use of anti-idiotypic antibodies as a means to purify soluble idiotypic T-cell receptors in rats. The idiotypic determinants were known to signify reactivity toward a given set of allo-MHC determinants (14–17). In such a system, it is known that low amounts of idiotypic, antigen-binding material can be isolated from the serum of normal adult rats allowing the purification of material from cell populations that have not undergone deliberate immunization procedures (14–17). We then used these purified T-cell receptor molecules to produce miniature immunosorbent columns that were tested for the ability to bind internally labeled, single MHC polypeptide chains of syngeneic or an allogeneic nature. Control columns included B-cell products (IgG alloantibodies) directed against the same allo-MHC structures or included normal serum IgG molecules.

By using this approach, we were able to demonstrate alloantigenic variability on single polypeptide chains of a MHC nature as demonstrated by selective retention on the immunosorbent columns. With the alloantibody columns it was thus possible to show retention of the major Ag-B chain as well as the two Ia chains if they were derived from the relevant allogeneic strain. No retaining ability for self-MHC or third-party MHC chains was noted with these types of columns. When using immunosorbents made up of idiotypic T-cell receptor material, only two MHC polypeptides of the relevant allo-MHC type were retained, namely, the Ag-B and the heavy Ia chains. However, the T-cell receptors could also be shown to display weak, but significant, reactivity toward one syngeneic MHC polypeptide chain, that is, the heavy chain of Ia type. At the same time, no detectable activity was observed when testing the same column for reactivity against third-party MHC polypeptide chains. The theoretical implications of these findings will be discussed.

Materials and Methods

Animals. Rats of the inbred strains Lewis (Ag-B^1), BN (Ag-B^3), and DA (Ag-B^4) as well as F_1 hybrids between these strains were bred and maintained in our own colony. Young adults of either sex were used in the experiments.

Lymphoid-Cell Suspensions. Spleens and lymph nodes were removed and single-cell suspensions were prepared in phosphate-buffered saline (D-PBS) by forcing through a stainless steel mesh. Large particles were removed by sedimentation and single cells were washed once in D-PBS by centrifugation for 8 min at 500 *g*. Erythrocytes were lysed by hypotonic shock using 0.9 ml of distilled water followed by 0.1 ml of 10-times-concentrated D-PBS. Cells were washed once more and resuspended in D-PBS. Dead cells were determined by trypan blue exclusion.

Preparation of Alloantisera. Sera of specificity Lewis anti-DA, Lewis anti-BN, and DA anti-Lewis were prepared in the following way. Each host was initially primed with a full-thickness skin graft (18). 3 wk after rejection, animals were boosted i.p. at monthly intervals with 5×10^7 allogeneic spleen cells. After five injections, animals were bled to death 10 d after the last

injection. Sera were heat-inactivated (30 min at 56°C) and stored at −20°C.

Preparation of Anti-Idiotypic Immunosorbent. An anti-idiotypic immunosorbent of specificity anti-(Lewis anti-DA) was prepared in (Lewis × DA)F_1 rats by injecting 2.5 × 10^7 Lewis T lymphocytes, eight times in 3-wk intervals. Animals were bled 6 d after the last injection and the serum pool (pool 3) was heat-inactivated and stored at −20°C. The anti-idiotypic nature of this antiserum has been published before (19). The Ig fraction of this antiserum was purified by dialysis against 15% Na_2SO_4 and coupled to cyanogen bromide (CNBr)-activated Sepharose 4B (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) as described before (15).

Purification of Lewis Anti-DA T-Cell Receptor Molecules. The procedure was described in detail before (15). Briefly, normal Lewis rat serum, which contains low amounts of idiotypic, alloantigen-binding T-cell receptor molecules (14, 15), was absorbed on the anti-idiotypic immunosorbent. After extensive washing by using D-PBS, bound material was eluted by glycin-HCl buffer, pH 2.8, followed by 2 M $MgCl_2$, 3 M urea, and 3 M guanidine-HCl. Eluted material was neutralized, dialyzed against concentrated D-PBS, and stored at −70°C.

Some eluted material was iodinated with ^{125}I by the chloramine-T method as described before (20). Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) analysis of iodinated material under reducing conditions on a 10% gel showed that the material contained a single polypeptide chain with a mol wt of ≅70,000 signifying idiotypic determinants, as well as alloantigen-binding, as described in detail before (14–16). Iodinated molecules corresponding to the peak of 70,000 daltons were eluted from the sodium dodecyl sulfate (SDS) gels by 2% SDS as described before (15). Such eluted molecules were then used as tracer molecules for the isolation of unlabeled T-cell receptor molecules from normal serum via SDS-PAGE. Molecules corresponding to 70,000 daltons were eluted from SDS gels and passed over Sephadex G-25 (Pharmacia Fine Chemicals, Inc.) in D-PBS in order to eliminate most of the SDS. Examination of such isolated molecules on SDS-PAGE after iodination showed us that the preparation contains highly purified T-cell receptor molecules without contamination of large amounts of other molecules. (See also Results: the purification and characterization of idiotypic Lewis anti-DA T-cell receptor molecules.) Purified material consisting of purified 70,000-dalton molecules was coupled to CNBr-activated Sepharose 4B.

1,000 ml of normal Lewis serum was absorbed on the anti-idiotypic immunosorbent. Approximately 4 mg of protein could be eluted from the immunosorbent. 600 μg of purified Lewis anti-DA T-cell receptor molecules could be isolated therefrom via SDS gels and were coupled to 500 mg of Sepharose 4B.

Preparation of Alloantibody Immunosorbent. Alloantisera of specificity Lewis anti-DA, Lewis anti-BN, and DA anti-Lewis were prepared as described above. Their selective activity with regard to binding to the corresponding spleen cells was demonstrated in the protein A assay (22, 23). To demonstrate anti-Ia-like activity of such alloantisera, they were absorbed with the corresponding purified erythrocytes. Erythrocytes were harvested from the blood, washed four times, and the contaminating lymphocytes were removed by centrifugation against Ficoll-Paque (Pharmacia Fine Chemicals, Inc.). 0.5 ml of alloantiserum was absorbed six times with 0.5 ml of packed, purified erythrocytes for 30 min at 4°C. Sera which still contained binding activity toward spleen cells, but no longer toward erythrocytes, were considered to contain anti-Ia-like antibodies (23–25). Unabsorbed antisera containing both anti-Ag-B serum-defined (SD) as well as anti-Ia lymphocytes-defined (LD) antibodies were used for the immunosorbents. The Ig fraction of such Lewis anti-DA, Lewis anti-BN, and DA anti-Lewis sera were coupled to CNBr-activated Sepharose 4B (15).

Preparation of Internally Labeled Ag-B and Ia determinants. Lewis, DA, and BN spleen cells were stimulated for 3 d with lipopolysaccharide (LPS). 15 ml of a cell suspension containing 2 × 10^6 cell/ml were incubated in Eagle's high amino acid (EHAA) medium (26) complemented with 1% fetal calf serum (FCS) and 10 μg/ml of LPS. LPS was a gift from Dr. Jan Andersson (Institute for Immunology, Biomedical Center, Uppsala University, Uppsala, Sweden). On day 3, cells were harvested and washed once in D-PBS and blasts were purified on Ficoll-Paque. 2 × 10^7 purified blasts were incubated for 6 h at 37°C in tissue-culture Petri dishes (No. 3002, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) in minimal essential medium (MEM) without leucine, 0.5 mCi of [^3H]leucine (sp act, 54 Ci/mmol), 0.5 mCi of [^3H]mannose (sp act, 2.7 Ci/mmol), 0.5 mCi of [^3H]galactose (sp act, 12.4 Ci/mmol), and 0.5

mCi of [^{3}H]fucose (sp act, 3.0 Ci/mmol). All tritiated reagents were purchased from the Radiochemical Centre, Amersham, England. Cells were washed four times and incubated again for 4 h in EHAA medium complemented with 1% FCS. Cells were then finally washed once more.

Preparation of Cell Lysate from Internally Labeled LPS Blasts. 2×10^7 internally labeled LPS blasts were lysed with 500 μl of buffer containing 0.05 M Tris-HCl, pH 7.6, 0.025 M KCl, 0.05 M NaCl, and 1% (wt/vol) Nonidet P-40 (NP40) (Fluka AG, Buchs, Switzerland) at 4°C for 30 min. Nuclei and cell debris were removed by centrifugation for 10 min at 20,000 *g*. Lysates were dialyzed overnight against the same buffer containing, however, only 0.5% NP40, and 1 mM phenylmethylsulfonylfluoride, 0.1% ϵ-amino-*n*-caproic acid, 0.1 mM tosyllysin chlormethylketone and 1 mM EDTA. Lysates were centrifuged for 5 min at 130,000 *g* in a Beckman Airfuge (Beckman Instruments Inc, Spinco Div., Palo Alto, Calif.). The supernates were kept at −70°C. 0.5 ml of supernate was absorbed on a 0.7- × 5-cm alloantibody-immunosorbent column. E.g., lysate from internally labeled DA blasts was absorbed on a Lewis anti-DA alloantibody-immunosorbent column. Likewise, supernates from Lewis blasts were absorbed on DA anti-Lewis and lysates from BN blasts were absorbed on Lewis anti-BN immunosorbents. Supernates were allowed to react with the immunosorbents for 15 min at room temperature and the columns were thereafter extensively washed with D-PBS. Bound material was eluted with high ionic strength (2 M $MgCl_2$, 3 M urea, and 3 M guanidine-HCl). Eluted material was dialyzed against D-PBS containing 0.5% NP40 and 100 μl was applied on SDS-PAGE. Molecules forming peaks of ≅44,000, 34,000, and 27,000 daltons were eluted from the first gel and separately rerun on a second SDS-PAGE. The corresponding single peaks were eluted again with 2% SDS and eluted material was passed over Sephadex G-25 in D-PBS containing 0.5% NP40. Purified molecules were immediately used for further experiments. Molecules used for functional studies with regard to binding to T-cell receptor molecules or corresponding alloantibodies were iodinated with ^{125}I by the chloramine-T method (20).

SDS-PAGE Analysis. 100 μl of material to be analyzed was boiled in 100 μl of 0.15 M 2-mercaptoethanol in 0.5 Tris buffer, pH 6.8, 10% glycerol, 0.1% SDS, and 6 M urea for 2 min and then applied on SDS gels (7). Electrophoresis for the analysis of tritiated samples was carried out in gels containing N,N′-diallyltartardiamide instead of N,N′-methylenebisacrylamide (8). After electrophoresis, gels were frozen and sliced into 1-mm slices. Slices containing tritiated samples were dissolved in 300 μl of 2% periodic acid before counting and counted in 2 ml of Instagel (Packard Instruments Co., Inc., Downers Grove, Ill.). Slices containing iodinated material were counted in a Intertechnique gamma counter (model CG-4000, Intertechnique Corp., Plaisir, France) equipped with a 3-in well-type crystal.

Results

Purification and Characterization of Idiotypic Lewis Anti-DA T-Cell Receptor Molecules. It has been shown previously that idiotypic Lewis T-cell receptor molecules with specificity for DA alloantigens can be isolated from normal Lewis serum by the use of an anti-(Lewis anti-DA) immunosorbent (15). Such molecules carry neither detectable Ig determinants of constant type nor structures which are under the control of the Ag-B complex (15–17). That these molecules are indeed produced by T lymphocytes could be shown by internal labeling procedures (15). The same method has been used in this study to produce Lewis anti-DA-specific T-cell receptor immunosorbent. 1,000 ml of normal Lewis serum was absorbed on an anti-(Lewis anti-DA) immunosorbent and bound material was eluted with low pH and high ionic strength. Eluted material was iodinated with ^{125}I and separated under reducing conditions on SDS-PAGE. Iodinated molecules forming a peak of ≅70,000 daltons were eluted from the gel and used as marker for the isolation of unlabeled T-cell receptor molecules on SDS-PAGE. Again, molecules with a mol wt of ≅70,000 daltons were eluted from the gels, passed over Sephadex G-25 and stored at −70°C. Gentle iodination of samples of such molecules, followed by a rerun on SDS-PAGE under reducing conditions resulted in

TABLE I

Iodinated Lewis Anti-DA T-Cell Receptor Molecules Absorbed on Various Preparations

Preparation	Input (cpm of ^{125}I)	Bound (cpm of ^{125}I)	Percentage
			%
Anti-(Lewis anti-DA) anti-idiotypic immunosorbent	100,000	86,237‡	(86.2)§
DA spleen cells*	20,000	17,414 ± 532‖	(87.1)
BN spleen cells*	20,000	2,559 ± 131‖	(12.8)
Lewis spleen cells*	20,000	4,117 ± 81‖	(20.6)

* 1×10^7 spleen cells in 100 μl of normal syngeneic rat serum were incubated with 20,000 cpm, representing the input = 100%, of purified ^{125}I-labeled Lewis anti-DA T-cell receptor molecules. Incubation was carried out for 2 h at 4°C.

‡ The binding of iodinated Lewis anti-DA T-cell receptor molecules onto anti-(Lewis anti-DA) anti-idiotypic immunosorbent was assessed by counting the eluted material (see Materials and Methods). 11.4% of the labeled molecules were passing the immunosorbent. 100,000 cpm represents the input = 100%.

§ Numbers in parentheses represent the percentage of the input.

‖ Values represent mean ± SE of quadruplicates.

a single peak of ≅70,000 daltons without significant contamination in higher or lower molecular-weight range. As shown previously, these molecules represent Lewis T-cell receptor molecules that can recognize DA alloantigens (15).

However, to ensure that the isolated molecules had retained their biological activity, the following experiments were carried out.

First, iodinated T cell receptor molecules were reabsorbed on anti-(Lewis anti-DA) anti-idiotypic immunosorbent. 86% of the material absorbed on the column could be eluted from the immunosorbent, whereas 11% of the molecules were passing the column. No significant binding was observed on rabbit anti-rat Ig, DA anti-Lewis, or normal rat Ig control columns, indicating that the majority of the isolated molecules carry idiotypic determinants of Lewis anti-DA type.

Second, iodinated receptor molecules were incubated with DA, BN, and Lewis spleen cells. We could first demonstrate that most of the SDS had been removed, as no lysis of the spleen cells occurred. The binding results of these experiments are shown in Table I. Approximately 87% of the Lewis anti-DA T-cell receptor molecules were absorbed by DA spleen cells, whereas 13% of the input was removed by BN spleen cells and ≅20% by Lewis spleen cells.

However, it has to be stressed that the iodinated T-cell receptor molecules tend to degrade very rapidly within the first 24 h and to loose their biological activity. The intensity of iodination seems to be the crucial factor but we do not know the exact reasons for the rapid degradation.

In summary we, could demonstrate that the Lewis anti-DA T-cell receptor molecules isolated from normal Lewis serum via different purification steps still carried idiotypic determinants as well as alloantigen-binding ability. 600 μg of these highly purified T-cell receptors were coupled to 500 mg of CNBr-activated Sepharose 4B and used a T-cell receptor immunosorbent.

Purification of Rat Single-Chain Molecules of MHC Type and Their Absorptions on Alloantibody Immunosorbent. Rat alloantigens were internally labeled and purified by the use

of alloantibody immunosorbents and SDS gels as described in Material and Methods. E.g., internally labeled DA alloantigens derived from LPS blasts were absorbed on Lewis anti-DA alloantibody immunosorbent (Ig fraction). The immunosorbent contained both anti-Ag-B as well as anti-Ia antibodies (Material and Methods). As a result of previous studies on Ag-B-coded determinants on rat lymphocytes, we had the information that Ag-B determinants can be found on SDS gels in the position of ≅44,000 daltons, and the heavy and light chains of Ia chains at ≅34,000 and 27,000 daltons (23). Indeed, internally labeled material derived from DA LPS blasts eluted from Lewis anti-DA alloantibody immunosorbent formed pronounced peaks in these regions, besides containing contaminating larger and smaller molecules (Fig. 1). Molecules forming the peaks at ≅45,000, 34,000, and 27,000 daltons were eluted from the gels and rerun separately on SDS gels. Respective peaks were again eluted from the gels, passed over Sephadex G-25, and stored at −70°C.

Molecules used for later experiments were iodinated and immediately used. Similarly, Ag-B and Ia chains were isolated and purified from BN and Lewis LPS blasts. Absorption experiments showed us that the isolated molecules could be bound onto the corresponding alloantibody immunosorbent (Table II). As shown, all three DA MHC chains (44,000-, 34,000-, and 27,000-dalton preparations) could be separately absorbed and eluted from Lewis anti-DA alloantibody (Table II) immunosorbent but failed to bind to Lewis anti-BN or DA anti-Lewis columns. Similarly, all BN MHC chains were bound on Lewis anti-BN immunosorbent and all Lewis molecules on DA anti-Lewis immunosorbent. No significant radioactivity could be eluted from the control columns. The experiments shown in Table II also contained tests of the molecules passing the immunosorbent or eluted from them as analyzed on SDS gels (data not shown). The profiles obtained demonstrated that the molecules that passed the column or that were bound and eluted from them were still intact as to their size.

These experiments gave us two pieces of information. Firstly, we conclude that the molecules purified as described above still express the corresponding epitopes although they underwent several purification steps, making selective binding to the corresponding alloantibody possible. No significant binding to control alloantibodies could be found. Secondly, it seems that alloantigenic specificities are expressed on both Ia chains (34,000 and 27,000 daltons), as the two separate chains did bind to the corresponding alloantibody.

Absorption of Purified Allo- or Self-MHC Chains on Lewis Anti-DA T-Cell Receptor Immunosorbent. Lewis anti-DA T-cell receptor molecules were purified and tested as described above and then coupled to CNBr-activated Sepharose 4B. MHC single chains of DA, BN, and Lewis type were purified as described above and absorbed on the T-cell receptor immunosorbent. The procedure was essentially the same as described in the legend to Table II. Instead of using a 0.7- × 5-cm column, we used a 0.7- × 4-cm column. 100,000 cpm of iodinated MHC molecules in 300 μl D-PBS were allowed to react with the immunosorbent for 30 min at room temperature. The columns were then extensively washed and bound material was eluted with 3 ml of a solution containing 2 M $MgCl_2$, 3 M guanidine-HCl, and 3 M urea. The results of such experiments (a total of 4) is shown in Table III. The Lewis anti-DA T-cell receptor immunosorbent was able to strongly bind Ag-B chains of DA origin (44,000 daltons) as well as the heavy Ia chain (34,000 daltons) of DA. The light chain (27,000 daltons) of DA Ia antigens, however, was not absorbed by the T-cell receptor

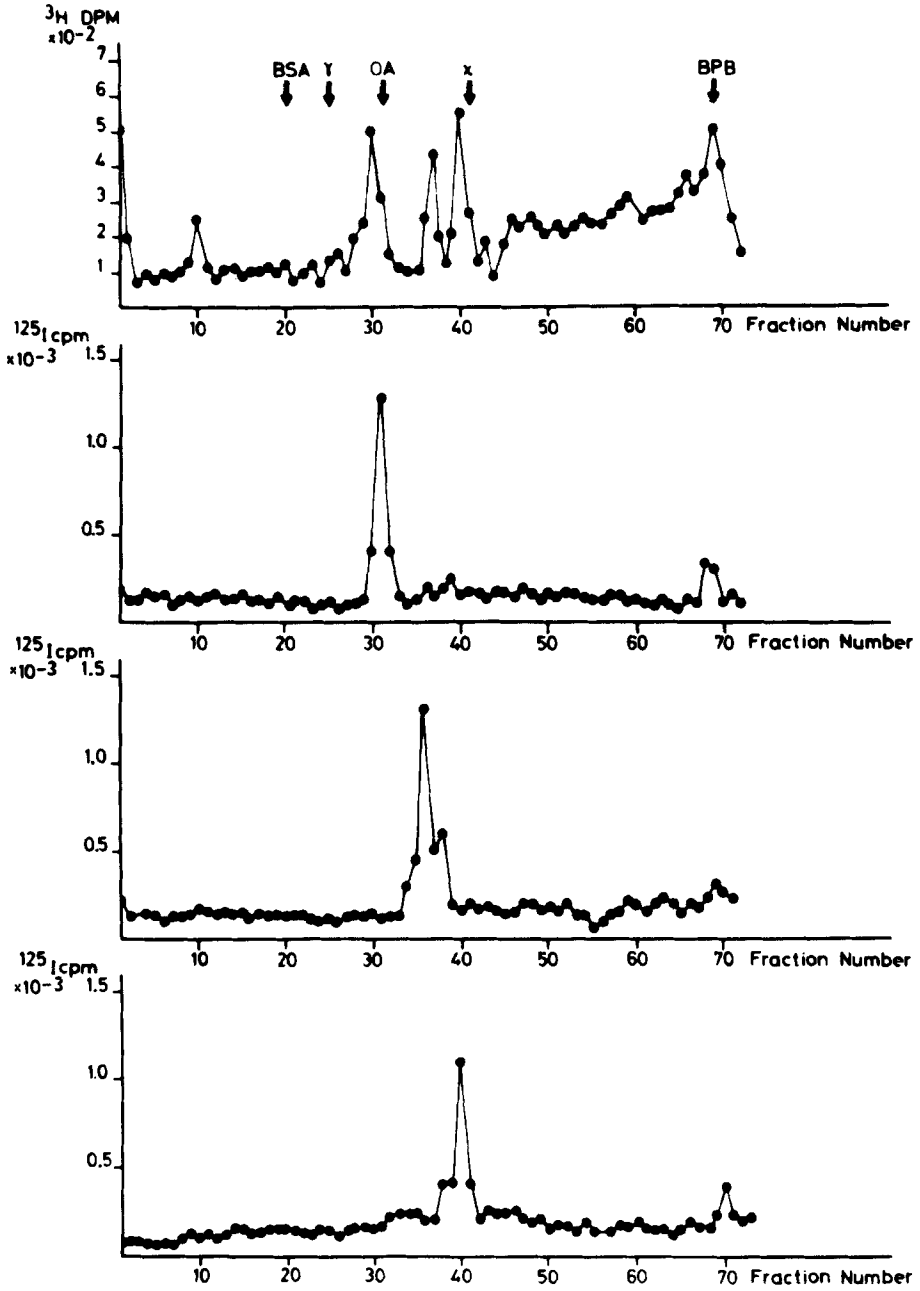

FIG. 1. DA spleen cells were stimulated with LPS and purified blasts were internally labeled with tritiated leucine and different sugars as described in Material and Methods. Supernate of cell lysate was absorbed on a Lewis anti-DA alloantibody immunosorbent and bound material was eluted with solutions having high ionic strengths and analyzed on 10% SDS gels. The top profile represents internally labeled material eluted from the immunosorbent. Molecules forming the peaks at mol wt of 44,000 (Ag-B chain), 34,000, and 27,000 (heavy and light chain of Ia) were eluted from gels and subsequently iodinated with ^{125}I and separately rerun on SDS gels. The corresponding profiles are shown. The molecules representing the above-mentioned molecular weights were eluted from the second gels and used for further experiments. In exactly the same way, Ag-B chains and both polypeptides of Ia of BN and Lewis lymphocytes were prepared.

TABLE II

Absorption of SD and LD Determinants on Different Alloantibody Immunosorbents

Immunosorbent	DA alloantigens			BN alloantigens			Lewis alloantigen		
	44K*	34K	27K	44K*	34K	27K	44K*	34K	27K
Lewis anti-DA									
Molecules passed	12,237 (12.2)‡	8,514 (8.5)	10,799 (10.8)	92,174 (92.2)	91,850 (91.9)	89,605 (89.6)	92,813 (92.8)	96,921 (96.9)	94,488 (94.5)
Molecules eluted	85,647 (85.6)	88,639 (88.6)	84,171 (84.2)	4,331 (4.3)	4,856 (4.9)	5,840 (5.8)	2,488 (2.5)	1,689 (1.7)	2,027 (2.0)
Lewis anti-BN									
Molecules passed	92,613 (92.6)	88,799 (88.8)	93,198 (93.2)	8,855 (8.9)	9,188 (9.2)	11,083 (11.1)	90,279 (90.3)	92,415 (92.4)	91,728 (91.7)
Molecules eluted	4,372 (4.4)	6,816 (6.8)	1,049 (1.1)	88,130 (88.1)	88.090 (88.1)	87,495 (87.5)	3,549 (3.5)	2,558 (2.6)	2,092 (2.1)
DA anti-Lewis									
Molecules passed	89,270 (89.3)	91,194 (91.2)	89,805 (89.8)	93,042 (93.0)	87,710 (87.7)	86.815 (86.8)	13,800 (13.8)	14,898 (14.9)	10,048 (10.0)
Molecules eluted	4,030 (4.0)	2,365 (2.4)	3,261 (3.3)	2,328 (2.3)	4,092 (4.1)	3,681 (3.7)	84,793 (84.8)	82,746 (82.7)	84,601 (84.6)

* SD (44K, 44,000 daltons) determinants as well as LD (34K, 34,000 daltons and 27K, 27,000 daltons) determinants were purified from DA, BN, and Lewis LPS blasts as described in Materials and Methods. 100,000 cpm representing the input = 100%, corresponding to 0.4 ml of each sample, was added to the immunosorbents and allowed to stand for 30 min at room temperature. Under these conditions the immunosorbents were by no means saturated. Material was eluted D-PBS containing 0.5% NP40, with phenol red as a control. All material was collected until phenol red was eluted. The columns were then washed again with 10 ml of D-PBS containing 0.5% NP40 which was discharged. Bound material was eluted with high ionic strength and low pH with a total vol of 5 ml and washed out with D-PBS containing phenol red. Material was collected until phenol red was washed out. Material passing the column and eluted from the columns was counted in a gamma counter.

‡ Numbers in parentheses represent the percentage of the input.

TABLE III

Absorption of Purified, Soluble Alloantigens on Lewis Anti-DA T-Cell Receptor Immunosorbent

Alloantigens	Molecules passed	Molecules eluted	Washing fluid
DA			
44K	8,379 (16.8)*	37,608 (75.2)*	939 (1.9)*
34K	5,756 (11.6)	40,321 (80.6)	1,103 (2.2)
27K	39,241 (78.4)	4,442 (8.9)	1,025 (2.0)
BN			
44K	43,018 (86.0)	4,291 (8.6)	874 (1.7)
34K	40,998 (82.0)	4,325 (8.6)	590 (1.2)
27K	39,633 (79.2)	6,474 (13.0)	483 (0.9)
Lewis			
44K	41,817 (83.6)	4,495 (9.0)	882 (1.7)
34K	7,357 (14.8)	8,236 (16.4)	30,283 (60.6)
27K	46,419 (92.8)	2,119 (4.2)	203 (0.4)

50,000 cpm of ^{125}I of alloantigens representing the input = 100%, corresponding to 0.3 ml, were added to the immunosorbent and allowed to stand for 30 min at room temperature (column not saturated). Material was washed out with D-PBS containing 0.5% NP40, with phenol red as a control. All material was collected until phenol red was eluted. The column was then washed with 10 ml of D-PBS containing 0.5% NP40 and the washing fluid was collected. Bound material was eluted with high ionic strength and low pH with a total vol of 5 ml and washed out with PBS containing phenol red. Material was collected until the phenol red was out. Material passing the column, washing fluid, and eluted material was counted in a gamma counter.

* Numbers in parentheses represent the percentage of the input.

immunosorbent. None of the three BN MHC chains could be shown to be bound onto the Lewis anti-DA T-cell receptor immunosorbent under the same conditions. Ag-B and the light Ia chain of Lewis type were not bound by the T-cell receptor immunosorbent. However, the heavy-chain group of Lewis Ia type with a mol wt of 34,000 was selectively retarded in its passage through the column. This led, in the beginning of this series of experiments, to the complete loss of radioactivity as the delayed molecules were discharged with the 10 ml of D-PBS washing fluid.

The experiment shown in Table III was completed by running the passed and eluted concentrated material over SDS gels (data not shown). It was found that such molecules were intact as judged by size corresponding to the expected relevant MHC chains.

These experiments showed us that Lewis anti-DA T-cell receptor molecules are able to bind DA Ag-B chains as well as the heavy DA Ia chain very strongly, but in contrast to the Lewis anti-DA alloantibody (Ig), the T-cell product was found to be unable to bind the light chain of DA Ia type. Most significantly, however, we found that Lewis anti-DA T-cell receptor molecules have a significant affinity to the self-MHC heavy chain of Ia type although they fail to bind third-party BN MHC chains.

Discussion

The present results provide new information at several levels. Firstly, the results obtained confirm and extend the earlier findings, in the mouse (29), that MHC molecules can be purified using SDS-PAGE conditions and recovered still maintaining

alloantigenic determinants. Here we could extend this to also include isolated single MHC-derived polypeptide chains when separated under reducing conditions in SDS, proving that these chains do have the ability by their own to re-nature in a significant manner. Using the approach with internally labeled, single chains of MHC-origin we could show that Lewis anti-DA alloantibody immunosorbent columns will retain DA MHC chains of Ag-B type as well as both heavy and light Ia type. No ability of the same columns to retain or retard MHC chains of Lewis (self) or BN (third-party MHC) was observed. As the MHC origin of the presently investigated polypeptide chains is well proven (23–25) the above data do demonstrate that alloantigenic variability in the rat can be shown to be expressed at both Ia chains, a matter of dispute in other species (30, 31).

However, the most intriguing new discoveries were made using immunosorbent columns made up of idiotypic Lewis anti-DA molecules isolated from normal Lewis serum in manners previously described (15). That such molecules are derived from Lewis normal T lymphocytes has been amply documented in earlier studies (15–17). Using such T-cell receptor columns, a strong binding ability for the relevant allo-MHC chains of DA type was noted; but here, in contrast to the Lewis anti-DA alloantibody immunosorbent, only two chains were retained, namely, the Ag-B and the heavy chain of Ia type. No measurable affinity for the DA light-chain Ia polypeptide was thus noted. This may suggest that T lymphocytes with specificity for MHC-coded determinants may be predominantly (or exclusively) reactive toward antigenic determinants present on only two of four types of polypeptide chains constituting the two major groups of MHC antigenic molecules. In support for such a view are findings obtained in MLC assays, where again in the same rat species, a selective ability of specific T blasts to bind heavy Ag-B or heavy Ia chains only was observed (32). It should however be realized that the present failure of the T-cell receptor immunosorbent column to remove light chain Ia molecules of DA type may merely represent a technical failure. Thus, the purification procedure of the T-cell receptor material involved the use of anti-idiotypic antibodies which may well, for reasons of variations, have failed to contain anti-idiotypic antibodies specific for the hypothetical T anti-light chain-Ia-determinant-specific receptors. It is, in fact, now well known that such a heterogeneity does exist at the level of T-cell idiotypes in allo-MHC situations as most clearly shown in the mouse where Lyt-1 and Lyt-2 positive blasts derived from the very same MLC can be shown to express distinctly different idiotypes (33).

Besides expressing a more selective binding to DA allo-MHC chains than the corresponding alloantibody immunosorbent columns, it was also found that the T-cell receptor immunosorbent had a wider reactivity when analyzed with regard to anti-self-MHC affinities. Thus, it could be clearly shown that the idiotypic Lewis anti-DA T receptors were also able to express significant affinity for one self-MHC chain, namely the heavy Ia chain of Lewis type. This was not evident by complete retention but was shown by a significant delay in passing through the immunosorbent column. As no such binding was noted when testing the same column for binding ability to third-party BN MHC chains, we must conclude that a bias toward self-MHC reactivity does indeed exist in the alloreactive T-cell receptor pool. It is clear that this is in nice agreement with the concept that T lymphocytes during their differentiation are indeed selected with regard to self-MHC reactivity (9, 10, 34). Our T-cell receptor

material could also be shown, when in soluble form, to express a very high degree of specific binding to the relevant allogeneic DA spleen cells (see Table I), where binding to >87% was noted. As these binding experiments were carried out using ^{125}I-labeled T-cell receptor molecules (that is, using externally labeled molecules known to be slightly damaged during labeling), we deem the present results to be sufficiently clearcut to conclude that the very same allo-MHC-reactive T-cell chains can also express a weak, but significant, selective binding ability for self-MHC determinants. We do not consider the fact that only reactivity against heavy self-Ia chain was noted in these experiments to be a conclusive finding excluding the possibility that a similar, self-Ag-B reactivity may also exist on the anti-allo-Ag-B-specific T-cell receptor level. This is stated because we have no estimates as to the relative proportions of anti-Ia versus anti-Ag-B receptors in the T-cell receptor columns, but data from other workers would suggest a relative predominance of anti-Ia when selecting material from normal T cells (35). Repetition of the present experiments using purified anti-allo-Ag-B-receptor immunosorbent columns would necessary, in this case, to solve this theoretically important question. The present results also further confirm our earlier observations that single chains of T-cell origin can indeed express strong binding ability in a specific manner to the relevant antigen. If the V_H-region on the corresponding alloantibody molecules are identical to that present on these T-cell receptor molecules, one would then assume that a similar binding ability would exist at the level of the single, isolated Ig-alloantibody chain. Also, such chains carrying similar or identical idiotypes as being present on the T-cell-derived chains would be expected to express a similar degree of self-MHC binding ability (which may be partly blocked by the V_L-regions normally associated with V_H in the B-cell-derived molecules). We are now attempting such studies.

In addition, the finding that the T-cell receptor chains are able to express significant binding to self-Ia chains does raise the intriguing possibility that the association between Ia molecules and antigen-specific, idiotypic T-cell factors noted by several workers (36–38) may have, as an underlying basis, forces created by a variable polypeptide regions of a clonal type. The failure of present results to find any measurable binding affinity to third-party MHC chains would thus, in our minds, exclude any strong binding ability resulting from noncovalent nature of the present class of T-cell-derived molecules. If the above suggestion is correct, one would thus expect that F_1 hybrid T cells on a single-cell basis would only produce antigen-specific factors associated with only one of the two possible MHC chains. The availability of functional T-cell hybridomas should facilitate such an experimental analysis.

Summary

In this study, we tried to get information about the fine antigen-binding ability of purified, soluble, idiotype-positive T-cell receptor molecules. Lewis anti-DA T-cell receptors were purified from normal Lewis serum by the use of anti-idiotypic immunosorbent and sodium dodecyl sulfate-polyacrylamide gel, and were coupled to cyanogen bromide-activated Sepharose 4B. In parallel, Lewis anti-DA, Lewis anti-BN, and DA anti-Lewis alloantibody immunosorbents were prepared. The major Ag-B chain (44,000 daltons) and the two polypeptide chains (34,000 and 27,000 daltons) of Ia were purified from Lewis, DA, and BN lymphocytes and absorbent on the above-mentioned immunosorbents. We found that the major Ag-B chain as well as

the two Ia chains were bound to the alloantibody columns if they were derived from the corresponding allogeneic strain. No retaining ability for self-major histocompatibility complex (MHC) or third-party MHC chains was noted with the alloantibody immunosorbents. When using immunosorbents made up of idiotypic T-cell receptors, only two MHC polypeptides of the relevant allo-MHC type were retained, namely, the Ag-B and the heavy Ia chains. No detectable activity was observed when testing the same column for reactivity against third-party MHC polypeptide chains. However, the Lewis anti-DA T-cell receptors could be shown to display weak, but significant, reactivity toward one Lewis MHC polypeptide chain, that is, the heavy chain of Ia type.

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