THE MAJOR HISTOCOMPATIBILITY COMPLEX-RESTRICTED ANTIGEN RECEPTOR ON T CELLS

I. Isolation with a Monoclonal Antibody*

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In recent years, many interesting results have been reported by investigators attempting to identify antigen receptors on T cells, but there has been limited success in the characterization of these molecules. Most efforts have focused on T cells or T cell products that have been demonstrated to bind free antigen (Ag), because in these cases an easily definable function could be used to assay any likely candidates that were identified. Using material of this kind, it has been suggested that T cell Ag receptors bear determinants that are cross-reactive with antibody idiotypes or V_H (1–12) and which also map to the major histocompatibility complex (MHC)[1] (7, 13–16). In addition, antisera raised against surface proteins on T cells, especially suppressor T cells, have suggested that determinants mapping near *Igh* are displayed on the surfaces of some types of T cells and are intimately involved with Ag recognition by these cells (17–19).

T cell products have been isolated using their ability to bind Ag and the various antisera mentioned above, and thus some preliminary biochemical data are available concerning these products. These proteins, usually suppressor factors, have molecular weights of ~70,000 (20–23), although smaller molecules have been reported (16). The 70,000-mol wt proteins have been shown to be susceptible to proteolytic cleavage into smaller subunits of 25–30,000 and 45–50,000 mol wt (20–23); the larger fragment possesses apparent functional activity and the smaller subunit may be the Ag-binding portion, although this may not always be so. The Ag-binding protein of 70,000 has been demonstrated to be a soluble factor secreted by suppressor T cells, but has also been identified in association with other T cell populations. The role of MHC products in Ag recognition by these structures is not clear.

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[1] *Abbreviations used in this paper:* BSS, balanced salt solution; cOVA, chicken ovalbumin; Con A, concanavalin A; HAT, hypoxanthine-aminopterin-thymidine; HgG, human gamma globulin; IL-2, interleukin 2; jfOVA, jungle fowl ovalbumin; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; qOVA, quail ovalbumin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; [³H]TdR, tritiated thymidine; tOVA, turkey ovalbumin; (TG)AL, poly-L(Tyr, Glu)-poly-D,L-Ala-poly-L-Lys; wOVA, widgeon ovalbumin.

Biochemical experiments dealing with MHC-restricted receptors for Ag (Ag/MHC receptors) on T cells have been less fruitful. Although there are some reports that these are structurally analogous to receptors that bind free Ag (6), this is by no means universally acknowledged. Our own experiments, and those of others (24–26), suggest that recognition of MHC products and Ag does not occur via independent receptors on T cell surfaces. Although there are suggestions that part of these receptors may map near *Igh* (27), we have failed to inhibit Ag/MHC recognition with anti-V_H reagents, and moreover, Ag/MHC receptors do not seem to be encoded on chromosomes coding for light chains or the MHC itself (28; and N. R. Roehm, K. Karjalainen, J. Kappler, and P. Marrack, manuscript in preparation). To resolve this dichotomy, we set out to study T cell receptors for Ag/MHC in a more direct fashion by raising monoclonal antibodies against these receptors on cloned T cell hybridomas. We have reported elsewhere (29) on the properties of antisera raised in congenic mice against Ag/MHC-specific T cell hybridomas. These antisera were determined to be highly specific in their ability to block Ag/MHC recognition by the immunogen T cell hybrid but not by other closely related T cell hybridomas.

In this paper we describe a monoclonal antibody obtained from spleen cells of a mouse that had produced high titers of inhibitory antisera. Like the antiserum of the mouse from which it was derived, this monoclonal antibody interferes with Ag/MHC recognition by the immunizing T cell hybridoma, but no other. The molecule recognized by this monoclonal antibody has an apparent molecular weight, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, of 80–90,000 under nonreducing conditions and of 40–44,000 after reduction, which suggests that a molecule with these biochemical properties is closely involved in Ag/MHC recognition by T cells.

Materials and Methods

Mice. All mice were bred in our facilities at National Jewish Hospital from breeding stock originally obtained from various individuals and suppliers as follows: BALB/cBy, AKR/J, C57BL10/SgSn, B10.A, and DBA/2J, The Jackson Laboratory, Bar Harbor, ME; BALB.B, Dr. Michael Bevan, Scripps Clinic and Research Foundation, La Jolla, CA; C.B20, Dr. Ian Zitron, Thomas Jefferson University, Philadelphia, PA.

Culture Conditions. All cultures were performed in modified Mishell-Dutton medium (24, 30) supplemented with 10% fetal calf serum and 5×10^{-5} M 2-mercaptoethanol.

Antigens. Chicken (c) and turkey (t) ovalbumin (OVA) and human gamma globulin (HgG) were obtained from the Sigma Chemical Co., St. Louis, MO. Poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(TG)AL] was obtained from Miles Laboratories Inc., Elkhart, IN. Jungle fowl (jf), quail (q), and widgeon (w) OVA were prepared in our laboratory by standard techniques (31) from eggs obtained from the Denver Zoo and CQF Manufacturing Co., Savannah, GA.

T Cell Hybridomas. The T cell hybridomas used in these studies were produced and characterized as previously described (24, 32). Briefly, T cell blasts enriched in antigen-specific cells were fused to an azaguanine-resistant T cell tumor (usually BW5147) using polyethylene glycol 1540. Hybrids were selected in hypoxanthine-aminopterin-thymidine (HAT)-containing medium and screened for their ability to secrete interleukin 2 (IL-2) when stimulated with antigen in the presence of Ag-presenting cells of the appropriate H-2 type. Hybrids with high reactivity were cloned at limiting dilution. The antigen and *I* region specificities of the hybrids were determined using several antigens and Ag-presenting cells from a bank of H-2 congenic and recombinant mice. Specificities were confirmed by inhibition of antigen presentation with monoclonal anti-Ia antibodies. Table I lists the cloned T cell hybridomas used in these studies with their relevant properties. The T cell hybridoma DO-11.10 responded to cOVA/I-A^d and cross-reacted with jfOVA/I-A^d and cOVA/I-A^h. Although three T cell hybridomas shown in

Table I share with DO-11.10 the property of recognizing cOVA/I-A^d, their receptors are clearly different, as they show different fine specificities when their ability to respond to various OVA and I-A products is measured.

IL-2 Production and Assay. The ability of T cell hybridomas to be stimulated to produce IL-2 was assessed as previously described with a few modifications (24, 28, 32). Briefly, 250-μl cultures were prepared containing limiting numbers of hybridoma T cells (1.25 × 10^4/culture), 100–200 μg of antigen, and either 1 × 10^6 irradiated (4,000 rad from a ^{137}Cs source) spleen cells or 1 × 10^5 A20-2J BALB/c B lymphoma cells (33, 34) as Ag-presenting cells. After 24 h the culture supernates were assayed in 100-μl twofold dilutions for the presence of IL-2 using the IL-2-dependent T cell line HT-2 (kindly provided by Dr. James Watson, University of Auckland, New Zealand) (35). A unit of IL-2 was defined as that amount present in the first twofold dilution in which the viability of HT-2 dropped below 90% after 24 h. Results were reported as units of IL-2 per milliliter of culture supernate. 10 U/ml was the minimum amount detectable.

Immunizations. Mice were immunized with 2 × 10^7 irradiated (4,000 rad from a ^{137}Cs source) hybridoma T cells intraperitoneally in balanced salt solution (BSS). Immunizations were given weekly for 4–6 wk. Thereafter, immunizations were given biweekly and on alternate weeks the mice were bled and antisera assayed individually for putative anti-Ag/MHC receptor antibodies (see below). Animals selected for fusions were rested 4 wk before receiving a final immunization intravenously 3 d before fusion.

B Cell Fusion Procotol. Mice selected for fusions were killed 3 d after a final injection of the immunogen T cell hybridoma. Spleen cell suspensions were prepared, washed in BSS, and treated with anti-T cell serum and rabbit or guinea pig complement to deplete T cells (36). The remaining spleen cells were mixed with washed P3-X63 Ag8.653 myeloma cells in a ratio of ~5:1 spleen cells to P3. After pelleting, the mixed cells were resuspended over the course of 1 min in 1 ml of a 40% solution of polyethylene glycol 6000, prewarmed to 37°C. The mixture was incubated at 37°C for 1 min, warm medium was then added dropwise to a final volume of 20 ml, and the mixture was incubated for 10 min at 37°C. The fused cells were gently washed, resuspended in medium containing 4,000-rad-irradiated spleen cells (3 × 10^6), and then plated out into 8–12 96-well microculture plates. After 24 h HAT was added and thereafter the culture medium was changed about every 5 d. The culture supernatants from wells positive for hybrid growth were tested for the presence of putative anti-receptor antibodies as described below.

Assay for Antibodies against Ag/MHC Receptors. Antibodies from immunized animals or from the supernatants of B cell hybridomas were assayed for activity against Ag/MHC receptors on T cells by two methods (29). The first method assumed that anti-Ag/MHC receptor antibodies might mimic the action of Ag/MHC. Antibodies were therefore assayed for their ability to stimulate IL-2 production by Ag/MHC-specific T cell hybridomas in the absence of any other activation signal. So far, we have not identified any antibody of this type in antisera or

TABLE I
T Cell Hybridomas Used in These Studies

| T cell hybridoma | Fusion partners | | | | Specificity | |
| | Normal T cell | | | Tumor cell | Ag/Self-I | Other |
	Strain	Ag	H-2			
DO-11.10	BALB/c	cOVA	d	BW5147	cOVA/I-A^d	cOVA/I-A^b, jfOVA/I-A^d
3DO-36.6	BALB/c	cOVA	d	BW5147	cOVA/I-E^d	jfOVA/I-E^d, tOVA/I-E^d
3DO-54.6	BALB/c	cOVA	d	BW5147	cOVA/I-A^d	jfOVA/I-A^d
3DO-54.8	BALB/c	cOVA	d	BW5147	cOVA/I-A^d	jfOVA/I-A^d, qOVA/ I-A^d, I-A^f, I-A^s
4DO-11.7	BALB/c	cOVA	d	BW5147	cOVA/I-A^d	tOVA/I-A^d, wOVA/I-A^d
3DT-18.11	BALB/c	(TG)AL	d	BW5147	(TG)AL/I-A^d	None
AO-40.10	B10.A	cOVA	a	FS6-14.13	cOVA/I-A^k	I-A^b
AODH-3.4	DBA/2	HgG	d	AO-40.10	cOVA/I-A^k	I-A^b

TABLE II

Characteristics of Subclones of DO-11.10

T cell hybridoma	Approximate number of chromosomes	Units of IL-2 per milliliter in response to			Percent specific binding ± SE to OVA/I-A^{d*}
		OVA/I-A^d	OVA/I-A^b	Con A	
DO-11.10	74	>640	320	>640	42.5 ± 5.6
DO-11.10.3	69	<10	<10	>640	0.8 ± 1.8
DO-11.10.7	69	<10	<10	>640	−1.4 ± 1.7
DO-11.10.15	67	<10	<10	>640	0.7 ± 1.5
DO-11.10.24	68	>640	40	>640	48.0 ± 5.8

* Percent specific binding was evaluated by binding radiolabeled hybridoma T cells to adherent A20-2J cells (a BALB/c B cell lymphoma; 33) pulsed with cOVA. Negative controls included unpulsed A20-2J cells. Percent specific binding is defined in Materials and Methods.

hybridomas from immunized mice. The second method assumed that anti-receptor antibodies would block Ag/MHC recognition by T cells. Antisera and supernatants of B cell hybridomas were therefore included in 250-μl cultures containing 1.25×10^4 hybridoma T cells, Ag, and 10^5 Ag-presenting lymphoma B cells, and the ability of the antibodies to interfere with IL-2 production was thus measured. Mouse antisera were always titrated (29), and supernatants from primary B cell hybridomas were assayed at a final concentration of 40%.

Hybridomas throught to be making antibody against Ag/MHC receptors were expanded, retested, frozen away, and cloned, and the clones were reassayed. Thus far, we have screened ~3,000 primary hybridomas with this technique. One monoclonal antibody with anti-receptor activity has been identified. This antibody is described in this paper.

Binding Assay. The ability of T cell hybridomas to bind to Ag/MHC was measured in a 1-h binding assay recently developed in our laboratory.[2] Adherent Ag-presenting B cell lymphomas or hybridomas (37) were plated out in microculture wells overnight. They were then washed and pulsed with Ag for 4 h at 37°C. After washing the cultures to remove unbound material, T cells prelabeled with tritiated thymidine ([^{3}H]TdR) were added and incubated for 1 h at 37°C. Unbound T cells were washed gently from the cultures and specific binding was estimated by liquid scintillation counting of [^{3}H]TdR in the bound cells. In these experiments results were calculated as: percent specific binding = [(counts per minute bound to specific Ag/MHC − counts per minute bound nonspecifically)/(total counts per minute added − counts per minute bound nonspecifically)]. In assays for inhibition of binding, aliquots of antibody were added to cultures before T cell hybrids.

Production of Subclones of DO-11.10 Lacking Receptors for cOVA/I-A^d. DO-11.10 was continuously cultured for 12 wk. At this point, the continuously cultured line and a sample of the same hybridoma freshly thawed from frozen stocks were challenged with concanavalin A (Con A) or cOVA/I-A^d. Although both samples of DO-11.10 responded equally well to Con A, the continuously cultured line made markedly less IL-2 in response to cOVA/I-A^d than the freshly thawed hybridoma. The continuously cultured line was therefore recloned at limiting dilution. Subclones were assayed for their ability to secrete IL-2 in response to cOVA/I-A^d, cOVA/I-A^b, and Con A. They were also tested for their ability to bind to adherent monolayers presenting cOVA/I-A^d (see Binding Assay, above).[2] Some preliminary karyotyping was done.

Sample results obtained with four of the subclones are shown in Table II. DO-11.10 and its subclone, DO-11.10.24, responded well to cOVA/I-A^d, cOVA/I-A^b, and Con A. They also had the ability to bind to cOVA/I-A^d-presenting monolayers in a 1-h binding assay. The other three subclones, DO-11.10.3, DO-11.10.7, and DO-11.10.15, lacked the ability to secrete IL-2 in response to cOVA/I-A^d or cOVA/I-A^b. This was apparently because they had lost the ability to synthesize receptors for these moieties because they continued to secrete IL-2 in response to Con A, and also these subclones failed to bind to cOVA/I-A^d-bearing monolayers in the 1-h

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[2] Marrack, P., B. Skidmore, and J. W. Kappler. Binding of antigen-specific, *H-2*-restricted T cell hybridomas to antigen-pulsed adherent cell monolayers. Manuscript submitted for publication.

binding assay. Notably, loss of receptors did not seem to correlate with a tremendous loss of chromosomes. None of the subclones differed from each other by more than a few chromosomes.

Surface Labeling and Cell Lysis. Membrane proteins were radioiodinated as previously described (38–40). Briefly, cells to be labeled were washed three times with phosphate-buffered saline (PBS) or with BSS and resuspended to a concentration of 5×10^7 cells/ml. The washed cells were combined with 0.5 mCi ^{125}I-Na (specific activity, 17 Ci/mg; New England Nuclear, Boston, MA) and then transferred to a tube containing 50 μg of Iodo-gen (1,3,4,6-tetrachloro-3α,6α-diphenyl-glycoluril; Pierce Chemical Co., Rockford, IL). After 10 min at room temperature, cells were washed three times with PBS containing 10^{-2} M potassium iodide.

Cell lysates were prepared by resuspending the washed cells (5×10^7/ml) in lysis buffer containing 1% Nonidet P-40, 5×10^{-5} M phenylmethylsulfonylfluoride, and 0.05 M iodoacetamide in 0.1 M Tris, pH 7.4, incubating the lysate at room temperature for 10 min, and then centrifuging the lysate at 10,000 g for 20 min to remove nuclei and cellular debris. The extent of labeling was determined by measuring incorporation of the radioisotope into 10% trichloroacetic acid-precipitable material.

Immunoprecipitation. Radiolabeled material was incubated with monoclonal antibody or control serum, and the immune complexes were insolubilized by adsorption to protein A-bearing *Staphylococcus aureus* according to the method of Kessler (41). Immune complexes were washed extensively and then solubilized by addition of 200 μl of electrophoresis loading buffer, followed by boiling and centrifugation to remove the bacteria.

SDS-PAGE. Samples analyzed in the unreduced state were made 50 mM in iodoacetamide before boiling; for reduced samples, 2-mercaptoethanol (5% final concentration) was added. Samples were than electrophoresed on 10% polyacrylamide gels containing SDS according to the method of Laemmli (42). For two-dimensional SDS-PAGE, nonreduced samples were electrophoresed on a 5–10% gradient slab gel with 3% stacking gel in the first dimension. Strips were then cut out of the gel, placed in electrophoresis buffer with 2-mercaptoethanol, and after reduction were placed on a 10% gel to run in the second dimension (43, 44). Radiolabeled polypeptides were revealed by autoradiography.

Affinity Chromatography. Monoclonal antibodies (IgG$_{2a}$ murine subclass) were purified on protein A-Sepharose columns (45). Affinity chromatography matrices were prepared by chemically coupling the selected reagents to activated agarose beads (Pharmacia Fine Chemicals, Piscataway, NJ).

Preparation of ^{125}I-labeled Monoclonal Antibody. Monoclonal antibody was purified from ascites fluid of mice growing the antibody-secreting hybridoma KJ1-26.1 by binding to protein A columns (45). 100 μg of the eluted antibody was labeled with 1.0 mCi of ^{125}I by the chloramine T method (46, 47). The labeling efficiency was 72%. Just before use the labeled antibody was spun in a Brinkmann microcentrifuge (Brinkmann Instruments, Inc., Westbury, NY) to remove aggregates.

Results

Anti-Id Antisera to T Cell Hybridomas. In a previous paper (29), we have described the production of antisera in congenic strains of mice to receptor material on antigen-specific, *I* region-restricted T cell hybridomas. In summary, immunizations with the T cell hybridoma DO-11.10 (Table I), specific for cOVA/I-A^d and cross-reacting with cOVA/I-A^b, were carried out in three groups of mice: (BALB/c $\times$ AKR)F$_1$ animals, syngeneic to the hybridoma; (BALB.B $\times$ AKR)F$_1$ animals, differing from the hybridoma at *H-2*; and (C.B20 $\times$ AKR)F$_1$ animals, differing from the hybridoma at *Igh*. Mice were immunized multiple times and sera from individual animals were assayed for anti-receptor antibodies, the activity of which was determined by the ability of antisera to inhibit the production of IL-2 by the immunogen T cell hybridoma in response to Ag and Ag-presenting cells. Anti-receptor antibodies were produced in all three groups of mice, occurring with highest frequency and titer in the (BALB.B $\times$ AKR)F$_1$ animals. The inhibitory antisera were idiotypically specific as they blocked

the response of DO-11.10, but did not affect responses of closely related hybridomas with different specificities. The interpretation that the inhibitory antibodies are directed against the Ag/MHC receptor(s) on DO-11.10 was substantiated by the production of antisera against another T cell hybridoma, 3DT-18.11, specific for $(TG)AL/I-A^d$. Like the anti-DO-11.10 antibodies, these reagents only blocked the response of the immunizing T cell hybridoma and not closely related hybridomas.

Examples of these results obtained with the serum of one animal, (BALB.B × AKR)F_1 mouse A, immunized with DO-11.10 are shown in Table III. The antiserum from this animal completely blocked the response of DO-11.10 to $cOVA/I-A^d$. The anti-$cOVA/I-A^b$ response of the same T cell hybridoma was also inhibited, although somewhat less strikingly. The inhibitory antibodies were specific for a determinant unique to DO-11.10 because they had no effect on the responses of closely related T cell hybridomas with similar gross, but different fine, specificities for Ag/MHC.

Splenic B cells from this animal were used as fusion partners in an attempt to make monoclonal antibodies with the inhibitory properties of its antiserum. One such monoclonal producer was found and characterized as described below.

A Monoclonal Antibody to the T Cell Hybridoma DO-11.10.   KJ1-26 was the 26th hybrid well picked from the B cell fusion of spleen cells from (BALB.B × AKR)F_1 mouse A immunized with the T cell hybridoma DO-11.10. Supernatant from the primary culture of this hybrid completely inhibited the response of DO-11.10 to $cOVA/I-A^d$. The hybrid cells were therefore cloned by limiting dilution and the culture supernate from the cloned line KJ1-26.1 was titrated for inhibitory activity in the response of DO-11.10 to $cOVA/I-A^d$. Inhibition of IL-2 production could still be easily detected with as little as 1 μl of antibody-containing supernate in 250 μl of culture (Fig. 1). The cloned cells were injected intraperitoneally into pristane-treated, 500-rad-irradiated, BALB/c mice for the production of ascites fluid. As is also readily observable in Fig. 1, the ascitic fluid of KJ1-26.1 was ~1,000-fold more effective than culture supernatant in blocking the $cOVA/I-A^d$ response of DO-11.10.

Classification of KJ1-26.1 for immunoglobulin isotype was carried out by an

TABLE III

Properties of Antisera from a (BALB.B × AKR)F_1 Animal Immunized with DO-11.10

T cell hybridoma	Culture conditions		Units per milliliter of IL-2	
	Ag	Ag-presenting cells	Without antisera	With antisera*
DO-11.10	cOVA	H-2^d	320	<10
DO-11.10	cOVA	H-2^b	160	20
3DO-36.6	cOVA	H-2^d	160	80
3DO-54.8	cOVA	H-2^d	320	320
AODH-3.4	cOVA	H-2^a	160	80
3DT-18.11	(TG)AL	H-2^d	320	320

* Pooled antisera from various bleeds of (BALB.B × AKR)F_1 mouse A immunized with DO-11.10 were added at a final concentration of 2% to the IL-2 induction cultures.
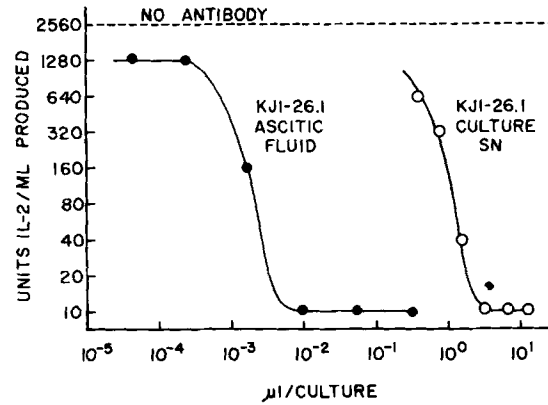
Fig. 1. Titration of inhibition of cOVA/I-A^d response of DO-11.10 by the monoclonal antibody, KJ1-26.1. Serial dilutions were made into microculture wells of either culture supernate or ascites fluid from KJ1-26.1. To each well were added DO-11.10 T cell hybrids (10^5), A20-2J-presenting cells (10^5), and 1 mg/ml OVA. After 24 h IL-2 production in the wells was assessed as described in Materials and Methods. The control response of DO-11.10 was 2,560 U IL-2/ml and is represented by the dotted line.

Ouchterlony analysis. Results showed that the monoclonal antibody was of the IgG$_{2a}$ subclass.

Specificity of KJ1-26.1 Monoclonal Antibody. In Fig. 2, results are shown from experiments run to demonstrate the specificity of the monoclonal antibody. Ascites fluid was diluted 1:1,000 and aliquots were placed in microculture wells with DO-11.10 or closely related hybridomas. Ag and appropriate Ag-presenting cells were then added and the resultant IL-2 production was measured. In contrast to the complete inhibition of DO-11.10 response to cOVA/I-A^d or cOVA/I-A^b, KJ1-26.1 had no effect on a hybridoma (AODH-3.4) with the same antigen specificity but different H-2 requirements, or a hybridoma (3DT-18.11) with comparable *I* region but different Ag specificity. Even more striking were the results obtained with various hybridomas with the same gross specificity for cOVA/I-A^d as DO-11.10 but with different fine specificities (Table I). In each case, no inhibition was seen in the presence of the KJ1-26.1 monoclonal antibody.

The fact that KJ1-26.1 only inhibited responses of DO-11.10 suggested that it was recognizing a determinant unique to this T cell hybridoma. Because IL-2 production by DO-11.10 in response to Con A was not inhibited, the antibody could not have been acting by suppression of IL-2 secretion. Moreover, KJ1-26.1 had no effect on the responses of other T cell hybridomas specific for combinations including cOVA or I-A^d, which proves that the antibody probably was not acting by binding to cOVA or I-A^d. The fact that I-A^d was not a target for KJ1-26.1 was further substantiated by the fact that DO-11.10 responses to cOVA/I-A^b were also blocked by this antibody. Thus, the most reasonable interpretation of these results was that KJ1-26.1 was specific for all or part of the receptors for cOVA/I-A^d and cOVA/I-A^b on DO-11.10.

Activity of KJ1-26.1 in Binding Assays. Although the experiments described in the previous section were most easily explained by the binding of KJ1-26.1 to Ag/MHC receptors on DO-11.10, we wished to test its activity more directly by measuring its
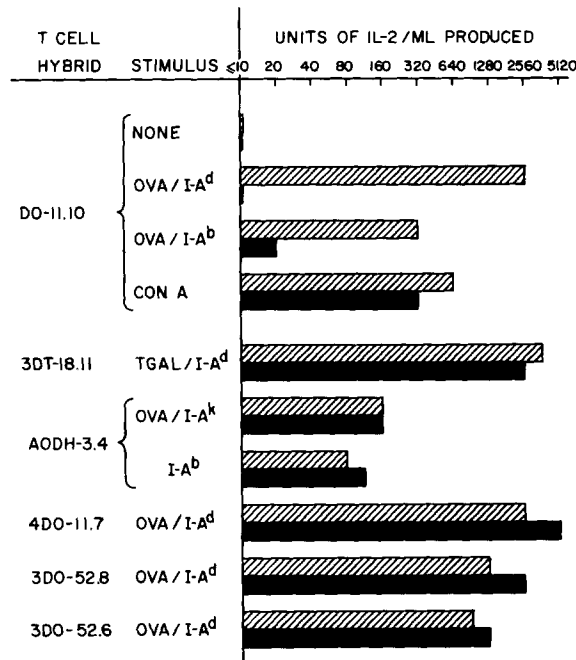
Fig. 2. Specificity of the monoclonal antibody KJ1-26.1. Cell cultures were prepared with or without antibody in microculture wells containing responder T cell hybrids, antigen, and antigen-presenting cells. The properties of the T cell hybrids used are shown in Table I. Presenting cells were: I^d, A20-2J; I^b, C57BL/10 spleen cells; I^k, B10.A spleen cells. In cultures being tested for inhibition of IL-2 production, KJ1-26.1 ascites fluid was used at a dilution of 1:1,000. Antibody-containing experiments are indicated by solid bars; cross-hatched bars represent controls without added antibody.

ability to interfere with the binding of DO-11.10 to cOVA/I-A^d. Radiolabeled DO-11.10, DO-11.10.24 (a cOVA/I-A^d-specific subclone of DO-11.10), and AODH-3.4 cells were added to microculture plates containing adherent monolayers of A20-2J (a BALB/c lymphoma; 33, 34) or LK4.5 (a B cell hybridoma bearing I^k and I^d; 37) prepulsed with cOVA, or LB15.13 (a B cell hybridoma bearing I^b and I^d; 37). Control wells contained lymphoma or hybridoma cells pulsed with HgG or in the case of AODH-3.4, A20-2J cells pulsed with cOVA. The assay is described in more detail in Materials and Methods.[2]

After 1 h incubation at 37°C, nonadherent cells were gently washed away, and the numbers of adherent DO-11.10 or AODH-3.4 cells were determined by scintillation counting. Results were calculated as percent specific binding ± standard error (SE) and are shown in Table IV. KJ1-26.1 profoundly affected the ability of DO-11.10 and DO-11.10.24 to bind to cOVA/I-A^d, but had no significant effect on the binding of AODH-3.4 to cOVA/I-A^k or I-A^b. These results suggest that the antibody was interfering specifically with Ag/MHC recognition by DO-11.10, i.e., that it was binding to the receptors for this complex on the T cell hybridoma.

Enumeration of Target Molecules for KJ1-26.1. Efforts were made to find out approximately how many target molecules for KJ1-26.1 were borne by each DO-11.10 cell. KJ1-26.1 antibody was coupled to ^{125}I as described in Materials and Methods. The radiolabeled antibody was then titrated into microculture wells containing DO-11.10

TABLE IV

Inhibition of Binding by KJ1-26.1

T cell hybridoma	Binding monolayer		Percent specific binding + SE*		Percent inhibition of binding
	Ag	H-2	Alone	With KJ1-26.1‡	
DO-11.10	cOVA	d	22.3 ± 4.6	1.6 ± 6.6	93
DO-11.10.24	cOVA	d	48.0 ± 5.8	5.0 ± 2.2	90
AODH-3.4	cOVA	k	23.5 ± 1.9	23.6 ± 4.7	0
AODH-3.4	—	b	33.0 ± 4.5	29.3 ± 4.3	11

* Percent specific binding is defined in Materials and Methods.
‡ KJ1-26.1 ascites fluid was added at a final concentration of 1% to binding assay wells.
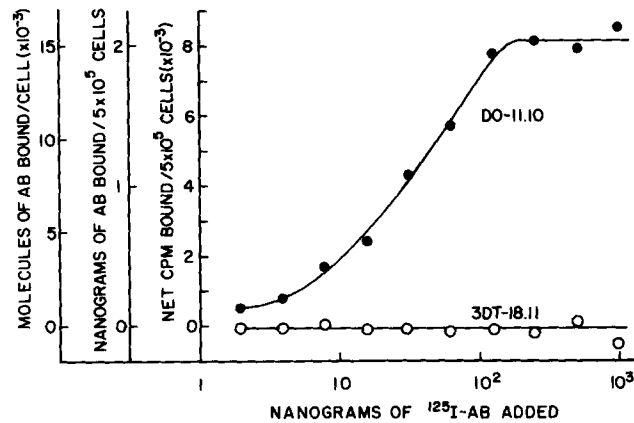
FIG. 3. Binding of ^{125}I-labeled KJ1-26.1 to DO-11.10 and 3DT-18.11. Microculture wells were prepared containing various amounts of ^{125}I-labeled KJ1-26.1 in 50 μl of BSS containing 25% fetal calf serum. Three identical sets of wells received 5×10^5 DO-11.10 (●), 5×10^5 3DT-18.11 (○), or no cells in 20 μl of BSS containing 25% fetal calf serum. After 2 h at 0°C, all wells were harvested using an automated cell harvestor (Otto Hiller, Madison, WI) on glass fiber filters (presoaked in fetal calf serum and washed thoroughly with saline). Filters were counted and the net counts per minute of ^{125}I-antibody bound to the cells was calculated, correcting for the counts per minute bound to filters from wells in which no hybrid cells were added (which was ~10% of the counts per minute bound to filters from wells containing DO-11.10 cells). A known quantity of the ^{125}I-labeled protein was counted and these counts were used to convert the scale of counts per minute both to nanograms of antibody bound per 5×10^5 cells and to molecules of antibody bound per cell.

or 3DT-18.11 cells. After incubation, cells were washed and harvested onto glass fiber filters. As shown in Fig. 3, there was no specific binding of KJ1-26.1 antibody to 3DT-18.11. In contrast, binding to DO-11.10 increased with increasing amounts of antibody added, reaching a plateau of 2 ng/5 $\times$ 10^5 cells. Results indicated that ~15,000 KJ1-26.1 antibody molecules were bound to each DO-11.10 cell, which suggests that the number of target molecules on DO-11.10 was the same order of magnitude.

In a second series of experiments we checked the ability of ^{125}I-KJ1-26.1 antibody to bind to a series of T cell hybridomas. As shown in Fig. 4, KJ1-26.1 bound to DO-11.10 and somewhat less well to DO-11.10.24, a cOVA/I-A^d-specific subclone of DO-11.10. The antibody did not bind to DO-11.10.3, DO-11.10.7, and DO-11.10.15, three
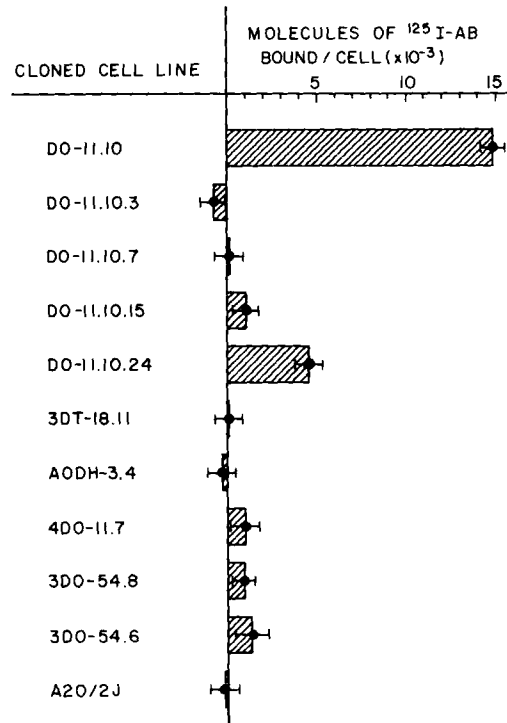
CLONED CELL LINE

MOLECULES OF ^{125}I-AB
BOUND / CELL $(\times 10^{-3})$

5   10   15

DO-11.10

DO-11.10.3

DO-11.10.7

DO-11.10.15

DO-11.10.24

3DT-18.11

AODH-3.4

4DO-11.7

3DO-54.8

3DO-54.6

A20/2J

Fig. 4. Idiotypic binding of ^{125}I-labeled KJ1-26.1. Microculture wells were prepared containing 300 ng of ^{125}I-labeled KJ1-26.1 as in Fig. 3. Triplicate wells received 5 × 10^5 of the 10 T cell hybridoma clones shown (the properties of which are indicated in Tables I and II). An 11th triplicate set received 5 × 10^5 A20-2J B lymphoma cells and a 12th set received no cells. Cultures were incubated, harvested on filters, washed, and counted as in Fig. 3. The net counts per minute bound was calculated and converted to molecules of ^{125}I-KJ1-26.1 bound per cell also as in Fig. 3. The results of this calculation are shown with the standard error.

subclones of DO-11.10 that had lost the ability to respond to or bind to cOVA/I-A^d or cOVA/I-A^b, but which retained the ability to secrete IL-2 in response to Con A (see Materials and Methods and Table II).

We concluded that the ligand of KJ1-26.1 was present only on cells bearing Ag/MHC receptors derived from DO-11.10. The ligand was not present on receptor-negative subclones of DO-11.10, or on other T cell hybridomas with clonally related, but not identical specificities (Table I). The putative Ag/MHC receptors were present at ~15,000/DO-11.10 cell and about 5,000/DO-11.10.24 cell. Other results, for example the Ag/MHC reactivity of DO-11.10.24 (Table II), had led us to suspect that DO-11.10.24 might bear fewer receptors per cell than DO-11.10.

Immunoprecipitation and SDS-PAGE Analysis of Molecules Reacting with KJ1-26.1. To identify the T cell surface protein(s) with which the monoclonal antibody KJ1-26.1 reacts, cell lysates of ^{125}I-labeled T cell hybridomas were incubated with aliquots of KJ1-26.1 ascitic fluid or in some cases with purified KJ1-26.1 antibody. The antigen-antibody complexes were harvested with formalin-fixed, protein A-bearing *S. aureus* and subsequently analyzed by SDS-PAGE. When the KJ1-26.1 immunoprecipitates of surface-labeled preparations of either DO-11.10 or 3DT-18.11 were analyzed by SDS-PAGE (10%), the patterns shown in Fig. 5 were consistently observed in six
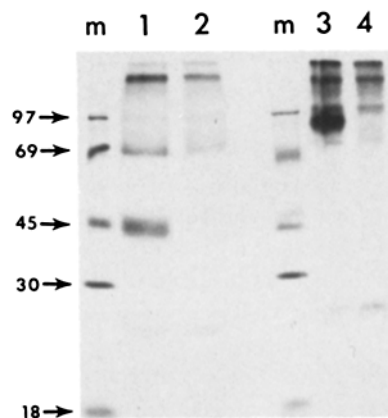
Fig. 5.   KJ1-26.1 immunoprecipitates of surface-labeled T cell hybridomas. DO-11.10 or 3DT-18.11 were surface-labeled with ^{125}I, lysed, and incubated with 25 µg of purified KJ1-26.1. Ag-antibody complexes were precipitated with protein A and electrophoresed on 10% SDS-PAGE gels under reducing (lanes 1 and 2) or nonreducing (lanes 3 and 4) conditions. Lanes 1 and 3 contain precipitates from DO-11.10, lanes 2 and 4 contain precipitates from 3DT-18.11. Molecular weight markers (m lanes) are as follows: 97 = phosphorylase B, 97,400; 69 = bovine serum albumin, 69,000; 45 = ovalbumin, 45,000; 30 = carbonic anhydrase, 30,000; 18 = lactoglobulin B, 18,400.
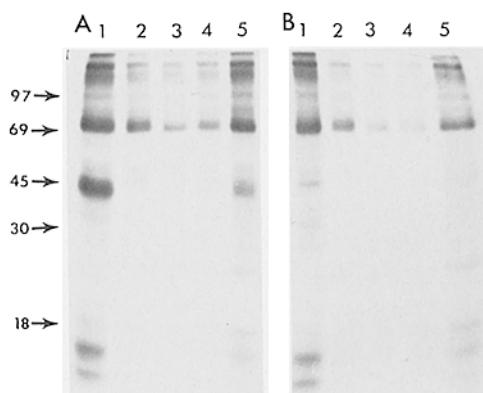

Fig. 6.   Immunoprecipitation of DO-11.10 or variant subclones by KJ1-26.1. Cell lysates were prepared from ^{125}I-surface-labeled DO-11.10 or variant clones of this T cell hybridoma. Immuno-precipitation was carried out using either the KJ1-26.1 ascites (A) or normal rabbit serum (NRS) (B), and samples were analyzed after reduction by SDS-PAGE on 10% gels and subjected to autoradiography. Material precipitated from DO-11.10 is shown in lane 1, and from its positive subclone, DO-11.10.24, in lane 5. Lanes 2-4 are patterns of immunoprecipitates from DO-11.10.3, DO-11.10.7, and DO-11.10.15, respectively, all of which are negative variants of DO-11.10.

separate experiments. Immunoprecipitates of DO-11.10 analyzed under reducing conditions were resolved into three major bands of radioactivity corresponding to molecules with apparent molecular weights of ~200,000, ~70,000, and 40-44,000, respectively (Fig. 5, lane 1). Under the same conditions, the KJ1-26.1 immunoprecip-itate of 3DT-18.11 lysates showed major banding in the 200,000 region, weak banding in the 70,000 region, and no product with an apparent molecular weight of 40-44,000. The 200,000 and 70,000 bands were observed in the SDS-PAGE patterns of nonspecific immunoprecipitates of both DO-11.10 and 3DT-18.11 lysates (data not shown, but see Fig. 6). Pretreatment of the lysates with staphylococci greatly reduced the

appearance of the 200,000 and 70,000 bands in the patterns of the specific immuno-precipitates. The 40–44,000-mol wt product obtained with the KJ1-26.1 immunopre-cipitation of DO-11.10 lysates was not influenced by the preclearance of the lysates with staphylococci.

When the same immunoprecipitates were analyzed without prior reduction with 2-mercaptoethanol, a band of radioactivity corresponding to an apparent molecular weight of 80–90,000 was observed in the KJ1-26.1 immunoprecipitate of DO-11.10 lysates but not of 3DT-18.11 lysates (Fig. 5, lanes 3 and 4). The bands in the higher molecular weight regions of the gel pattern seen in the immunoprecipitates of both T cell hybridomas were due to material nonspecifically immunoprecipitated. These results suggest that the KJ1-26.1-reactive T cell surface component of the DO-11.10 T cell hybridoma is an 80–90,000-mol wt protein composed of disulfide-bonded subunits of ~40–44,000.

The reactivity of the KJ1-26.1 monoclonal antibody on surface-labeled DO-11.10 and the subclones of this T cell hybridoma (Table II) were compared. In Fig. 6 (panel A) are illustrated the SDS-PAGE patterns of KJ1-26.1 immunoprecipitates (reduced with 2-mercaptoethanol prior to electrophoresis) of cell lysates of DO-11.10 (lane 1), an Ag/MHC receptor-bearing subclone, DO-11.10.24 (lane 5), and several receptor negative subclones (lanes 2–4). The 40–44,000 protein is readily observed in the patterns of the immunoprecipitates of DO-11.10 and DO-11.10.24 cell lysates, but is not detected in the immunoprecipitates from the lysates of the negative variants. Again, the bands of radioactivity observed in the high molecular weight region (i.e., >70,000) appear to represent material nonspecifically bound or trapped in the immune complexes as these products can be resolved in the control immune precipi-tates (Fig. 6, panel B).

Thus, the two T cell hybridoma lines that were known by binding and IL-2 production studies to bear receptors for cOVA/I-A^d were also able to bind KJ1-26.1 (Fig. 4) and yield 40–44,000-mol wt precipitates with the antibody, whereas the three cOVA/I-A^d nonreactive subclones of DO-11.10 were negative in all these assays, which strongly suggests that the 40–44,000-mol wt material represented all or part of the receptor(s) for cOVA/I-A^d on DO-11.10. Significantly less 40–44,000 material was precipitated by KJ1-26.1 from DO-11.10.24 than from DO-11.10 (Fig. 6). Binding and IL-2 studies had also suggested that the subclone bore fewer cOVA/I-A receptors per cell than DO-11.10.

Further resolution of the KJ1-26.1 immunoprecipitates from cell lysates was accom-plished by two-dimensional SDS-PAGE analysis (43, 44). For this purpose, samples in unreduced form were electrophoresed on a 5–10% gradient slab gel in the first dimension. Strips from this gel were excised, equilibrated in electrophoresis sample buffer containing 5% 2-mercaptoethanol, and then loaded onto a 10% slab gel and electrophoresed. Under these conditions, proteins that are covalently bonded by disulfide bonding can be identified in the second dimension as spots that do not lie on the diagonal. The diagonal pattern is formed by proteins that migrate in the same fashion with or without prior reduction with 2-mercaptoethanol (i.e., noncovalently associated products). Fig. 7 shows the two-dimensional gel patterns of KJ1-26.1 immunoprecipitated material from two separate experiments. In the first experiment, the immunoprecipitates from DO-11.10 (Fig. 7A) and 3DT-18.11 (Fig. 7B) were compared and in the second experiment, the immunoprecipitates of DO-11.10 (Fig.
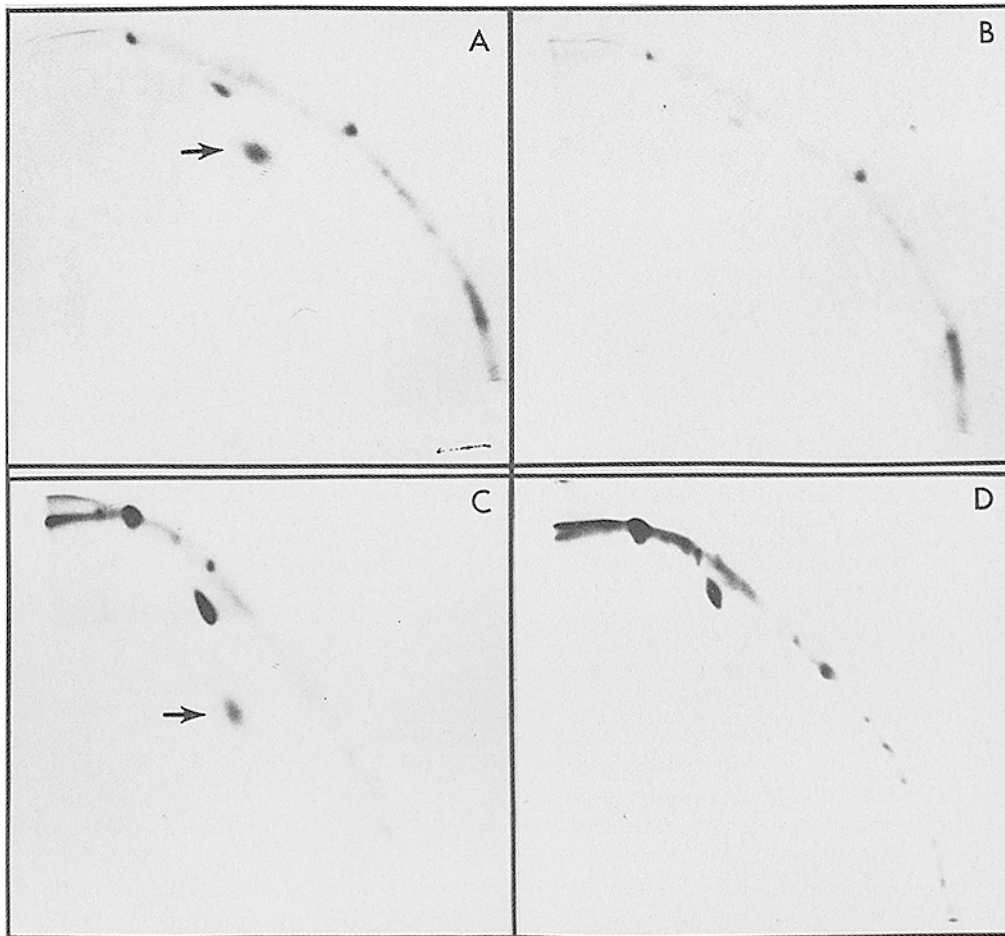
Fig. 7. Two-dimensional SDS-PAGE analysis of ^{125}I-labeled membrane proteins immunoprecipi-
tated with KJ1-26.1. T cell hybridomas were ^{125}I-surface-labeled, lysed, and precipitated with KJ1-
26.1 and protein A. Samples of each precipitate were electrophoresed in nonreduced form in the
first, horizontal, dimension and in reduced form in the second, vertical, dimension. The autoradi-
ograms shown represent the results of two separate experiments, the first in which KJ1-26.1
immunoprecipitates from DO-11.10 (A) and 3DT-18.11 (B) were compared, and the second in
which immunoprecipitates from DO-11.10 (C) or its negative subclone, DO-11.10.3 (D) were
compared. The arrows in A and C point to the 40–44,000 mol wt protein(s).

7C) and the negative variant DO-11.10.3 (Fig. 7 D) were compared. In both of the
patterns of the KJ1-26.1 immunoprecipitates of DO-11.10 cell lysates, a prominent
spot lying off the diagonal in a region corresponding to an apparent molecular weight
of 40–44,000 can be seen. The vertical position of this product (i.e., its relative
mobility in the first dimension) corresponds to an apparent molecular weight of 80–
90,000 as anticipated from the one-dimensional analysis of the unreduced and reduced
immunoprecipitates (Fig. 5). Neither the 3DT-18.11 nor the DO-11.10.3 immunopre-
cipitates shows the presence of the 40–44,000-mol wt product, although the proteins
forming the diagonal pattern are identical to those in the diagonal seen in the DO-
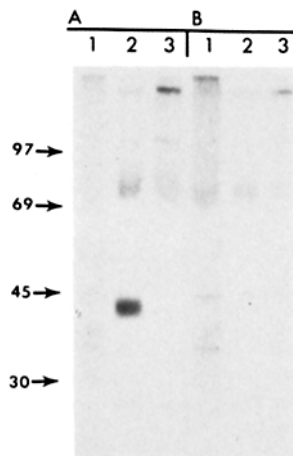11.10 immunoprecipitate patterns. The dark-intense spot of higher molecular weight

Fig. 8. Immunoprecipitation using KJ1-26.1-conjugated Sepharose beads. A B cell lymphoma and T cell hybridomas were ^{125}I-surface-labeled, lysed, and immunoprecipitated by Sepharose beads conjugated either to KJ1-26.1 (A) or to HOPC-1 (B). Specifically bound, eluted products were analyzed after reduction on SDS-PAGE. Autoradiogram patterns are as follows: lane 1, A20-1.11, a BALB/c B cell lymphoma (33); lane 2, DO-11.10; and lane 3, DO-11.10.3, the negative variant of DO-11.10.

that lies slightly off the diagonal is seen in all the gel patterns and is the viral glycoprotein gp70, since preclearance of the lysates with a rat anti-gp70 monoclonal reagent markedly reduces the appearance in subsequent KJ1-26.1 immunoprecipitates (results not shown). This gel system gives clear resolution of the covalent structure of the KJ1-26.1-reactive T cell component and its subunit structure. It is not clear whether the 40–44,000 product represents a single molecular species or multiple components of similar size, and therefore, whether the covalent form is a homodimer or a heterodimer.

Immunoprecipitations of radiolabeled cell lysates were also carried out using purified monoclonal antibody conjugated to Sepharose 4B beads. In Fig. 8 are shown the SDS-PAGE autoradiograms of the labeled materials from three different cell lines: A20-1.11, an Ia^d-expressing B cell lymphoma (33) (lane 1), DO-11.10 (lane 2), and DO-11.10.3, the receptor negative subclone of DO-11.10 (lane 3). Bound material was eluted from immunoadsorbents consisting of KJ1-26.1 antibody conjugated to Sepharose 4B (panel A), or HOPC-1, a γ_{2a} mouse myeloma protein, coupled to Sepharose 4B (panel B). The materials eluted from the HOPC-1 immunoadsorbent represent nonspecifically bound or trapped material and only in the case of radiolabeled products from DO-11.10 cell lysates eluted off the KJ1-26.1 immunoadsorbent is a distinct product resolved (i.e., the 40–44,000 protein). This species is not detected either in the material eluted from the KJ1-26.1 immunoadsorbent from A20-1.11 or DO-11.10.3 cell lysates or from the materials eluted from the HOPC-1 immunoadsorbent. These patterns are very similar to those obtained using purified antibody or ascitic fluid and protein A-bearing staphylococci, but less nonspecific binding material is consistently observed using the bead-coupled antibody reagents.

Discussion

One of the problems in identifying antibodies that react with T cell receptors for Ag/MHC has been the difficulty in unequivocally demonstrating that any given

reagent is actually reacting with Ag/MHC receptors rather than some other surface molecule. Because of the cumbersome nature of assays for binding of T cells to their Ag/MHC targets,[2] putative anti-receptor antibodies have often been assayed for their ability to stimulate or block the response of Ag/MHC-specific T cells. Several perhaps misleading and currently inexplicable reactivities have thus been discovered. For example, anti-Thy-1 antibodies often seem to stimulate T cells to produce IL-2 or divide (48), although there is no reason to suppose any relationship between Thy-1 and Ag/MHC recognition by T cells. Several reagents have been reported to block T cell responses. These include Lyt-2, and OKT8 or Leu-2, which block the functional activity of cytotoxic T cells in mouse and man, respectively (49–51), and OKT4 or Leu 3 (52), and a recently described equivalent in mouse (D. Wilde, D. Dialynas, and F. Fitch, personal communication), which blocks Ag reactivity of DR or I-restricted T cells in man or mouse. The mapping of Lyt-2 near K chains in mouse makes results with this Ag particularly intriguing. However, the fact that the targets of all these inhibitory antibodies do not seem to vary in amino acid sequence or antigenicity on different T cell clones and that some noninhibited T cell lines have been found suggests that these molecules do not actually comprise the variable portions of T cell receptors for Ag/MHC, though they may be intimately involved with these polypeptides.

The production of antibody that is clone specific in its ability to mimic or block Ag/MHC reactivity by T cells is therefore an encouraging feature, because this leads us to hope that the antibody in question is reacting with some clone-specific, "idiotypic" molecule on the T cell surface, presumably a part of its receptor. Several reports have been published of such clone-specific antibodies (53, 54) although no extensive biochemical studies have yet appeared. We have been producing clone-specific antibodies in congenic mice immunized with cloned Ag/MHC-specific T cell hybridomas; the monoclonal antibody KJ1-26.1 discussed in this paper is the product of a B cell hybridoma derived from of one of these mice.

There is every reason to suppose that KJ1-26.1 is reacting with all or part of the receptor(s) for Ag/MHC on the immunizing T cell hybridoma DO-11.10. The antibody blocks IL-2 production in response to Ag/MHC by this T cell hybridoma but no others. This includes T cell hybridomas of the same strain derivation (BALB/c fused to BW5147) and even of closely related but not identical specificities. The case would probably be strengthened by the use of another T cell hybridoma with the same fine specificity for Ag/MHC as DO-11.10. In this case we would predict that KJ1-26.1 might have blocking activity. Unfortunately, DO-11.10 represents a rather rare clonotype, defined by its cross-reaction with jfOVA/I-A^d and with cOVA/I-A^b. We have not yet observed a second independent T cell hybridoma with this fine specificity.

The idea that KJ1-26.1 reacts with a determinant expressed only by DO-11.10 was strengthened by binding and precipitation studies. The radiolabeled antibody failed to bind to any of a number of closely related T cell hybridomas or to precipitate material from any T cell hybridomas other than DO-11.10 and its Ag/MHC receptor-bearing subclone, DO-11.10.24. Thus, not only was KJ1-26.1 unable to inhibit responses to Ag/MHC by other T cell hybridomas, but the determinant recognized by this antibody was apparently absent from their surfaces.

Several other pieces of information suggested that KJ1-26.1 was binding to all or

part of the Ag/MHC receptor on DO-11.10. First, the antibody failed to block IL-2 production by DO-11.10 in response to Con A, which suggests that it interfered with Ag/MHC recognition rather than IL-2 secretion per se. Second, in 1-h binding assays the antibody blocked DO-11.10 and DO-11.10.24 binding to cOVA-pulsed I^d-bearing adherent monolayers, but had no effect on the binding to Ag/MHC of a control T cell hybridoma. Third, the fact that the antibody failed to block cOVA recognition by any of a panel of cOVA-specific T cell hybridomas besides DO-11.10 suggested that cOVA itself was not a target of the antibody. The immunization protocol used to raise KJ1-26.1 and its binding and immunoprecipitation properties of course also supported this idea. Fourth, KJ1-26.1 did not seem to be acting by binding to H-2-encoded determinants, either on DO-11.10, or on Ag-presenting cells. Because the antibody was derived from (BALB.B × AKR)F_1 cells immunized with a (BALB/c × AKR) T cell hybridoma, this was of course a possibility. KJ1-26.1 failed to bind or precipitate material from the H-2^d-bearing, Ia$^+$, Ag-presenting B cell lymphoma A20-2J, or any BALB/c-derived T cell hybridomas other than DO-11.10 and its derivative. Moreover, the molecular weight of the material precipitated from DO-11.10 was not similar to that of any known H-2 product of either class 1 or class 2 type. For example, no β_2-microglubulin was precipitated, and on unreduced gels, an 80,000-mol wt dimer was identified. The molecular weight of the precipitated material, and its unique expression on DO-11.10, also suggested that the antigen precipitated by KJ1-26.1 was not any of the molecules commonly found on T cells, T200, transferrin receptors, lymphocyte functional antigen-1, Lyt-1, Lyt-2, Thy-1, or the murine equivalent of OKT4 (55–58; D. Wilde, D. Dialynis, and F. Fitch, personal communication).

Finally, we were very encouraged to find that KJ1-26.1 failed to bind to or precipitate material from subclones of DO-11.10 that were thought to have lost the ability to synthesize receptors for cOVA/I-A^d or cOVA/I-A^b. Because these subclones failed to bind or respond to Ag/MHC but continued to secrete IL-2 in response to Con A, it was likely that they had lost receptor material, probably because of chromosome loss, a relatively common event in hybridoma cells of all types.

Taken together, these results strongly suggest that KJ1-26.1 reacts with the receptors for cOVA/I-A^d or cOVA/I-A^b on DO-11.10. There was therefore considerable interest in the material precipitated by this antibody. Our gel studies showed clearly that under nonreducing conditions, a band of ~80–90,000 mol wt was precipitated that migrated at 40–44,000 mol wt under reducing conditions. This was most dramatically illustrated by two-dimensional gel analysis in which this band and gp70 were the only radiolabeled surface proteins of DO-11.10 present in the immunoprecipitate to fall below the diagonal line. In every case, although most of our precipitates were contaminated to some degree by the major surface components known to be on T cells and T cell hybridomas, e.g., T200 and gp70 (55, 59), the specific precipitation of material from DO-11.10 by KJ1-26.1 could be easily observed. More recently, the use of KJ1-26.1 affinity columns has allowed cleaner isolation of the material of interest. The reason for the nonspecific precipitation of other T cell surface moieties with KJ1-26.1 was not entirely clear. Usually, specific precipitation enhanced nonspecific "trapping," which suggests that precipitation of membrane vesicles, for example, might be causing this effect.

Molecules of the type precipitated by KJ1-26.1 have been described before. Goding and Harris (60) described a disulfide-bonded diffuse spot on gels of radiolabeled

thymocytes that seemed to be associated with Lyt-2, but which had the molecular weight properties of the molecule we have identified. More recently, Allison et al. (61) have isolated a similar molecule from a C57BL/Ka x-ray-induced T cell lymphoma. Interestingly, and probably significantly, in their case the molecule was identified using a monoclonal antibody that reacted only with the molecule on the immunizing T cell, which suggests that it might have been reacting with a receptor (of unknown Ag/MHC specificity) on the surface of the tumor. In this study, the 80,000-mol wt dimer was shown to be made up of two chains of slightly different molecular weights (39,000-41,000) and different isoelectric focusing patterns. The covalent form of the molecule precipitated by KJ1-26.1 may well be composed of similar subunits, as suggested by the diffuse nature of the spots seen in gel patterns.

The homology between the molecule identified by KJ1-26.1 and other molecules on the surface of or secreted by T cells that bind free Ag is not obvious. In general, molecules of the latter type seem to be ~70,000 mol wt dissociating to 45,000 and 25,000 fragments. Several alternatives are possible. The 45,000 chain identified in Ag-binding material may be similar to one of the ~40,000 chains that make up the molecule we and others (60, 61) have isolated. Alternatively, since most previous work has involved Ag-binding receptors, and the receptors on DO-11.10 have no apparent affinity for cOVA in the absence of I-A^d or I-A^b, it is possible that two completely different types of receptors are being studied in these experiments. The apparent absence of V$_H$ determinants or MHC products in Ag/MHC receptors of our T cell hybridomas would support this (28; and N. R. Roehm, K. Karjalainen, J. Kappler, and P. Marrack, manuscript in preparation).

Many questions have yet to be answered in our studies. Does KJ1-26.1 identify the entire receptor(s) for Ag/MHC on DO-11.10 or is just a portion of the receptor(s) precipitated? Is the structure of the receptor(s) on DO-11.10 for Ag/I-A identical to the structure of receptors on other T cells specific for Ag/class I molecules or Ag/I-E? What are the differences in sequence between receptor material on DO-11.10 and on T cell lines of different specificities? These questions will occupy future work on the subject.

Summary

An antibody-secreting B cell hybridoma, KJ1-26.1, has been prepared from mice immunized with the T cell hybridoma DO-11.10, which recognizes chicken ovalbumin in association with I-A^d (cOVA/I-A^d). KJ1-26.1 blocks I-restricted antigen recognition by DO-11.10 and a subclone of this T cell hybridoma, DO-11.10.24, which has the same specificity for cOVA/I-A^d as its parent. KJ1-26.1 does not block I-restricted antigen recognition by any other T cell hybridoma tested, including a number of T cell hybridomas closely related to DO-11.10, with similar, but not identical, specificities for antigen/I. Moreover, KJ1-26.1 binds to DO-11.10 and DO-11.10.24, but not to any other T cell hybridomas tested, including three subclones of DO-11.10 that have lost the ability to recognize cOVA/I-A^d. Thus, in every regard KJ1-26.1 appears to be binding to all or part of the receptors for antigen/I on the T cell hybridoma DO-11.10.

KJ1-26.1 appears to bind to ~15,000 molecules/cell on the surface of DO-11.10. The antibody precipitates an 80,000 dimer from the cells, which on reduction migrates as 40-44,000 monomers. The receptor(s) for antigen/I on DO-11.10 therefore includes

molecules with these properties.

Note added in proof: Another molecule which is similar to that precipitated by KJ1-26.1 has come to our attention. Reinherz et al. (Reinherz, E. L., S. C. Meuer, and S. F. Schlossman. 1983. *Immunol. Today (Amst.).* **4:**5) have recently reported clone-specific monoclonal antibodies which precipitate glycoprotein chains of 49,000 and 43,000 mol wt from cloned human cytotoxic T cells.

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