Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 1820–1824, March 1996
Immunology

A recombinant antibody with the antigen-specific, major histocompatibility complex-restricted specificity of T cells

PETER S. ANDERSEN*, ANETTE STRYHN†, BJARKE E. HANSEN†, LARS FUGGER‡, JAN ENGBERG*§, AND SØREN BUUS†

*Department of Biological Sciences, The Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark; †Department of Experimental Immunology, Institute of Medical Microbiology and Immunology, University of Copenhagen, Blegdamsvej 3C, DK-2200 Copenhagen, Denmark; and ‡Department of Clinical Immunology, National University Hospital, Tagensvej 20, DK-2200 Copenhagen, Denmark

Communicated by J. J. van Rood, Academisch Ziekenhuis Leiden, Leiden, The Netherlands, November 1, 1995 (received for review June 14, 1995)

ABSTRACT **Specific recognition of peptide/major histocompatibility complex (MHC) molecule complexes by the T-cell receptor is a key reaction in the specific immune response. Antibodies against peptide/MHC complexes would therefore be valuable tools in studying MHC function and T-cell recognition and might lead to novel approaches in immunotherapy. However, it has proven difficult to generate antibodies with the specificity of T cells by conventional hybridoma techniques. Here we report that the phage display technology is a feasible alternative to generate antibodies recognizing specific, predetermined peptide/MHC complexes.**

T and B cells represent two fundamentally different recognition modes of the specific immune system. Through alternating selection processes T cells are educated to recognize antigenic peptides presented in association with self-molecules of the major histocompatibility complex (MHC) on the surface of antigen-presenting cells. In contrast, B cells are not educated to be self-MHC-restricted and B-cell receptors (antibodies), whether soluble or in membrane-bound form, recognize three-dimensional target structures. The distinctly different education of B and T cells explains why antibodies with the MHC-restricted specificity of T cells are rare and why it has been difficult to generate such specificities by conventional B-cell hybridoma techniques. We have taken advantage of the selection power of the phage display technology which makes it possible to test tens of millions of individual clones and have devised a method to generate recombinant antibodies recognizing predetermined peptide/MHC complexes. The speed and feasibility of this method makes it realistic to produce antibodies to a variety of specific peptide/MHC complexes which may be useful in studying MHC-restricted T-cell recognition and may lead to novel approaches in diagnostics and immunotherapy.

MATERIALS AND METHODS

MHC Purification. The AKR mouse-derived lymphoma RDM-4 was used for K^k production as described (1). In brief, K^k molecules were immunoaffinity purified from detergent cell lysates by using the monoclonal anti-K^k antibody 11.4-1 (American Tissue Type Culture Collection). The affinity columns were washed extensively and bound MHC class I molecules were eluted with 0.05 M diethylamine, pH 11/0.15 M sodium chloride/0.1% sodium azide/0.1% sodium deoxycholate, neutralized, and concentrated by vacuum dialysis. Human β_2-microglobulin was obtained from the urine of uremic patients and purified to homogeneity by gel filtration and chromatofocusing (1).

The influenza virus-derived nucleoprotein peptide NP_{50-57} (single-letter code, SDYEGRLI) and hemagglutinin peptide $Ha_{255-262}$ (FESTGNLI) were synthesized manually on a RaMPS synthesizer (DuPont) using standard fluorenylmethoxycarbonyl protection strategy.

Generation of Peptide/MHC Class I Complexes. Detergent-solubilized, affinity-purified MHC class I molecules (15 μM) were incubated at 18°C for 30–48 hr with NP_{50-57} or $Ha_{255-262}$ peptide (44 μM) in the presence of human β2-microglobulin (5 mM). The reaction mixture contained 1 mM phenylmethylsulfonyl fluoride, 8 mM ethylenediaminetetraacetic acid (EDTA), 1.2 mM 1,10-phenanthroline, 69 μM pepstatin A, 128 μM 7-amino-1-chloro-3-tosylamido-2-methylheptanone ("N^{α}-*p*-tosyl-L-lysine chloromethyl ketone"), 135 μM L-1-tosylamido-2-phenylethyl chloromethyl ketone ("N^{α}-*p*-tosyl-L-phenylalanine chloromethyl ketone"), and 1 mM *N*-ethylmaleimide in phosphate-buffered saline (PBS: 0.14 M sodium chloride/0.01 M sodium phosphate buffer). The final detergent concentration in the reaction mixture was 0.05% Nonidet P-40 and the pH was adjusted with a citrate buffer to pH 5.5. These conditions effected an almost complete exchange of K^k-bound peptides (data to be published elsewhere). Nonbound peptides were (in some experiments) removed by Sephadex G-25 spun column chromatography (2).

Peptide/MHC Complexes Displayed on Cells or Coated onto Beads. Complexes were mixed with 1.5×10^7 sulfate latex beads (diameter, 5 μm; Interfacial Dynamics) and incubated overnight at 4°C with gentle shaking. The remaining binding sites on the beads were blocked with 1% bovine serum albumin (BSA) in PBS. The beads were washed once and resuspended in 1 ml of FACS buffer (1% BSA and 0.1% sodium azide in PBS) and used for either panning or fluorescence-activated cell sorter (FACS) analysis. RMA-S·K^k cells (donated by V. Ortiz-Navarette, Mexico City) were grown overnight at 26°C (10^7 cells in 5 ml) in a medium containing 0.1 mM NP_{50-57} or $Ha_{255-262}$ peptide (see ref. 2). Cells were harvested by centrifugation, washed once, and suspended in 1 ml of growth medium.

Immunization. Inbred H-2^k (BALB/k) mice were primed with live attenuated *Mycobacterium tuberculosis bovis* (bacille Calmette–Guérin, BCG) and subsequently immunized with an aluminium hydroxide emulsion containing purified protein-derived peptide of tuberculin (PPD) covalently coupled with $Ha_{255-262}/K^k$ complexes. The antigenic solution was generated by mixing 0.5 mg of $Ha_{255-262}/K^k$ complexes in 0.5 ml of 0.1 mM phosphate buffer, pH 7.5/0.05% Nonidet P-40 with an equal volume of phosphate-buffered 0.2% glutaraldehyde solution and rotated end-over-end at 4°C for 2 days before addition of 20 ml of PBS containing aluminum hydroxide at 2 mg/ml. Three weeks after the BCG priming, mice were immunized twice with 0.5 ml of the antigenic mixture. The

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Abbreviations: BCG, bacille Calmette–Guérin; BSA, bovine serum albumin; FACS, fluorescence-activated cell sorter; IL-2, interleukin 2; PPD, purified protein-derived peptide of tuberculin.

§To whom reprint requests should be addressed.

immunizations were given subcutaneously with 2-week intervals. Spleens were collected 10 days after the second immunization.

Phage Display Library, Panning, and Purification of Soluble Fab Fragments. Total RNA from spleens of immunized mice was isolated and combinatorial Fab libraries displayed on phage were generated as described (3, 4). Our initial peptide/MHC library consisted of 1.6×10^7 independent clones.

For panning on cells, RMA-S·K^k cells pulsed with $Ha_{255-262}$ (2×10^5 cells in 80 μl) were mixed with 20 μl of phage library containing 10^{11} colony-forming units (cfu) and incubated at room temperature for 3 hr with gentle shaking. Cells were washed three times with growth medium and bound phages were eluted by trypsin treatment (3, 4). Cells were pelleted by centrifugation and the supernatant was transferred to vials containing 400 μl of exponentially growing *Escherichia coli* TOP10F′ cells (British Biotechnology) that were later superinfected with R408 helper phage (Stratagene) as described (3, 4).

For panning on beads, 1×10^6 latex beads coated with purified $Ha_{255-262}/K^k$ complexes were mixed with 20 μl of phage library containing about 10^{11} cfu in PBS/1% BSA and incubated for 3 hr at 4°C with gentle shaking followed by three washes with PBS/1% BSA. Bound phages were eluted after the final wash by treatment with 100 μl of 0.1 M glycine buffer/1% BSA, pH 2.2, for 10 min followed by addition of 8 μl of 2 M Tris base. The phages were processed as above.

The Fab coding cassettes of selected clones were transferred to an expression vector which adds a $(His)_6$ tag to the C terminus of the light chain in place of the gene III element. By using this new construct, Fab fragments were prepared from the periplasmic fraction (4, 5) and purified by Bakerbond ABx chromatography (J. T. Baker Research Products) followed by chromatography on chelating Superose HR 10/2 (Pharmacia) according to the instructions of the manufacturers.

FACS Analysis. Peptide-pulsed RMA-S·K^k cells (10^5) or peptide-coated beads (10^5) were mixed with Fab phages ($\approx 4 \times 10^9$ cfu/ml) or with soluble Fab13.4.1 and incubated on ice for 2 hr. Bound phages or Fab molecules were detected with fluorescein isothiocyanate-conjugated rabbit anti-M13 phage or rabbit anti-mouse IgG (Dako), respectively, and analyzed with a FACScan (Becton Dickinson) equipped with LYSIS II software.

Cytotoxic T-cell Assays. The generation of the class I-restricted mouse T-cell hybridomas HK9.5-24 and HK9.5-162 (NP_{50-57} specific, K^k-restricted) and HK8.3-5H3 and HK8.3-6F8 ($Ha_{255-271}$-specific, K^k-restricted) has been described (2). RMA-S·K^k cells were pulsed with NP_{50-57} or $Ha_{255-262}$ peptide and resuspended in culture medium in the presence of various concentrations of Fab13.4.1. T hybridoma cells (10^5) were incubated with 10^5 peptide-pulsed RMA-S·K^k cells for 24 hr at 37°C in a total of 250 μl and the supernatant was tested for interleukin 2 (IL-2) release by assay on the IL-2-sensitive cell line HT2, according to the procedure of Kappler *et al.* (6).

BIAcore Analysis. The concentration of formed complexes (i.e., active binding sites of K^k) to be used for calculations of the kinetic constants was determined by adding trace amounts of ^{125}I-labeled NP_{50-57} with known specific activity to purified complexes and determining complex formation by Sephadex G-25 spun column chromatography (2).

Purified Fab13.4.1 was diluted to 20 μg/ml in 10 mM acetate buffer, pH 4.5, and coupled to the dextran surface of BIAcore chips by standard amine coupling chemistry according to the manufacturer's recommendations (Pharmacia). The amount of Fab fragment coupled to the chips was kept just below 1000 resonance units (RU). Purified and quantitated peptide/MHC complexes were introduced into the measuring chamber and the flow speed was set to 10 μl/min to avoid interference of mass transport limitations with the kinetic calculations. All measurements were done in PBS containing 0.1% Nonidet P-40 at 22°C.

RESULTS

Complexes between the purified mouse MHC class I molecule K^k and the K^k-restricted influenza virus-derived peptide $Ha_{255-262}$ were generated (1, 2). For immunization purposes, PPD was coupled to the purified complexes and used to immunize BCG-primed H-2^k mice (BALB/k), which should be largely tolerant to K^k as a B-cell immunogen, but highly reactive to PPD as a T-cell immunogen. Syngeneic animals were chosen to favor the generation of an antibody response directed against self-MHC-restricted epitopes, and the BCG–PPD immunization regime originally described by Lachmann *et al.* (7) was chosen for its efficiency. Total spleen mRNA was isolated 10 days after the immunization and reverse transcribed to cDNA. Specific sets of degenerate primers (3) were used to PCR amplify the cDNA segments corresponding to the immunoglobulin Fab fragments, and the fragments were subsequently cloned into the pFab5c vector (3) and expressed in fusion with the minor viral coat protein pIII of the filamentous bacteriophage. The library was subjected to panning procedures followed by elution of bound phages and reamplification in *E. coli*. To enhance the efficiency of the selection procedure, phages were panned on K^k-transfected RMA-S cells (RMA-S·K^k) (2) pulsed with $Ha_{255-262}$, alternating with panning on latex beads coated with purified homogeneous $Ha_{255-262}/K^k$ complexes (2). The purpose of our panning strategy was to change the matrix for every other panning round while maintaining a common selecting epitope, $Ha_{255-262}/K^k$. Reamplified phages from each round of selection were adjusted to 4×10^9 cfu/ml and tested for binding to $Ha_{255-262}$- or NP_{50-57}-pulsed RMA-S·K^k cells by FACS analysis using fluorescein-conjugated anti-phage antibodies (the peptide NP_{50-57} peptide used for comparison is also K^k-restricted). As illustrated in Fig. 1, a progressive enrichment for Fab phages recognizing $Ha_{255-262}/K^k$ was observed after the 2×2 alternating rounds of panning. The relative small increase in binding to NP_{50-57}-pulsed RMA-S·K^k cells (solid bars) during panning rounds 1–3 probably reflects an increase in Fab phages binding with low affinity to common cell epitopes. This population was effectively removed as a result of the fourth and final panning round on beads.

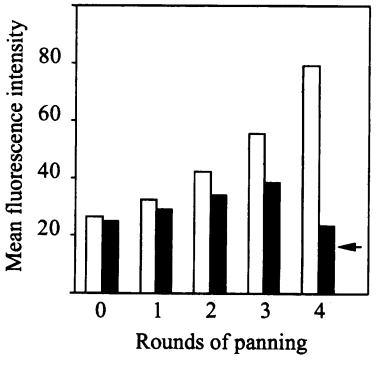

FIG. 1. Phage selection by biopanning. A library of phages expressing immunoglobulin Fab fragments was panned on alternating matrixes bearing $Ha_{255-262}$/MHC complexes as the only common denominator. Panning rounds 1 and 3 were on $Ha_{255-262}$-pulsed RMA-S·K^k cells, and panning rounds 2 and 4 were on beads coated with purified $Ha_{255-262}/K^k$ complexes. Bound phages were eluted and amplified in *E. coli* after each panning round and analyzed by FACS for binding to peptide-pulsed RMA-S·K^k cells. The peptides were $Ha_{255-262}$ (open bars) or NP_{50-57} (closed bars). The black arrow indicates the background signal without added phage.

Individual phages were isolated from the population of phages from the final panning round and rescreened for specificity by ELISA. Of 50 clones tested, seven reacted specifically with $Ha_{255-262}/K^k$ complexes. Fifteen clones bound to both $Ha_{255-262}/K^k$ and NP_{50-57}/K^k complexes and most likely recognized epitopes shared by the two complexes. The remaining 28 clones bound neither to $Ha_{255-262}/K^k$ nor to NP_{50-57}/K^k complexes. The DNA sequence corresponding to the heavy- and light-chain complementarity-determining regions 3 of the seven K^k-specific clones were determined and found to be identical (data not shown), suggesting that they were all derived from a single productive antibody light-chain/heavy-chain combinatorial event. Soluble Fab molecules from one of the clones, pSAN13.4.1, were produced and purified (Fig. 2*A*) and the specificity was assayed by FACS analysis using latex particles coated with various combinations of peptide and MHC. The specificity of the Fab molecule (Fab13.4.1) had the hallmarks of MHC-restricted T-cell specificity since reactivity was seen only for complexes formed between $Ha_{255-262}$ and K^k, neither component could be recognized alone, nor could irrelevant NP_{50-57}/K^k complexes be recognized (Fig. 2*B*). Furthermore, the interaction was saturable and specific as evidenced by the ability of soluble $Ha_{255-262}/K^k$ complexes—but neither isolated $Ha_{255-262}$ peptide, isolated K^k, nor NP_{50-57}/K^k complexes, to compete with bead-coated $Ha_{255-262}/K^k$ in binding to Fab13.4.1 (Fig. 2*C*). Ten to 100 nM soluble $Ha_{255-262}$-K^k complexes were needed to inhibit the interaction between Fab13.4.1 and $Ha_{255-262}/K^k$, indicating that the affinity, (the equilibrium dissociation constant, K_D) of Fab13.4.1 for $Ha_{255-262}/K^k$ complexes is between 10 and 100 nM.

Surface plasmon resonance (i.e., detection of changes in refractive index on a surface; BIAcore, Pharmacia) was used to confirm the specificity of Fab13.4.1 and to determine the kinetics of the interaction. Purified Fab13.4.1 was immobilized onto BIAcore sensor chips and challenged with preformed $Ha_{255-262}/K^k$ or NP_{50-57}/K^k complexes. As shown in Fig. 3*A*, the sensorgrams demonstrate binding of Fab13.4.1 to $Ha_{255-262}/K^k$, but not to NP_{50-57}/K^k. As a control, the K^k-specific monoclonal antibody H100-27R55 (8) was immobilized onto BIAcore sensor chips and challenged with the same preparations of peptide–K^k. H100-27R55 bound both peptide/K^k complexes equally well (Fig. 3*B*), thereby confirming that equal amounts of functional K^k protein were available to the chips. The slope of the initial binding phase depended on the concentration of $Ha_{255-262}/K^k$ (Fig. 3*C*), allowing us to calculate the association rate constant, k_a, to be 1×10^4 $M^{-1}\cdot s^{-1}$. The sensorgrams of Fig. 3*C* revealed two experimental problems: (*i*) a blank injection gave rise to an increase in signal of about 19 RU and a nearly linear decrease to about 3 RU at the end of the association time and (*ii*) the base line declined about 1 RU/min during the course of the experiments, indicating a slow loss of immobilized ligand. Both these experimental deviations were interpolated into straight lines and used to correct the association phases and the entire sensorgrams, respectively. These corrections and the following calculation were done by using mathematical models developed by Ron Shymko (personal communication). Twenty-one data points from each sensorgram were used for the mathematical analysis of both the dissociation and the association. First the dissociation phases were fitted by a double exponential model. The calculated dissociation rate constant (k_d) values were in the range of $7.3 \times 10^{-4}\cdot s^{-1}$ to $3.2 \times 10^{-4}\cdot s^{-1}$. Using these values and keeping the total number of binding sites constant, we fitted the entire sensorgrams to a two-site model. This gave k_a values for the high-association component in the range of 1.2×10^4 $M^{-1}\cdot s^{-1}$ to 0.6×10^4 $M^{-1}\cdot s^{-1}$, and the resulting K_D values were very similar: 53, 56, 60, and 51 nM. Parameters for the low-affinity component could not be interpreted. However, the contribution of this component to the sensorgram data was small, varying from 3% to 18% of the total signal at the end of the association.

Removal of unbound ligand initiated a dissociation phase which could be used to determine k_d as $5.6 \times 10^{-4}\cdot s^{-1}$ (corresponding to a $t_{1/2}$ of about 21 min). K_D, calculated as k_d divided by k_a, was found to be about 56 nM, a value that correlates well with the inhibition range observed in Fig. 2*C*. The kinetics and affinity of two different T-cell receptors determined by BIAcore analysis were recently published (9, 10). Association rate constants, k_a, of 10^3 and 10^5 $M^{-1}\cdot s^{-1}$ and dissociation rate constants, k_d, of 0.06 and 0.2 s^{-1} (corresponding to a $t_{1/2}$ of 12–27 s) were reported, leading to the calculation of overall equilibrium dissociation constants, K_D, of 10^{-5}–10^{-7} M. By comparison, antibodies generally associate with about the same rate as T-cell receptors, whereas they tend to dissociate orders of magnitude slower (11). Typically the overall affinities of antibodies are 10^{-7}–10^{-10} M. The affinity and kinetics of Fab13.4.1 are, despite its T-cell-like specificity, typical of an antibody.

Given the specificity and affinity of Fab13.4.1, we expected it to inhibit $Ha_{255-262}$-specific, K^k-restricted T-cell hybridomas, but not K^k-restricted T-cell hybridomas of different peptide specificity (2). Indeed, Fab13.4.1 could inhibit two $Ha_{255-262}$-specific, K^k-restricted T-cell hybridomas, HK8.3-5H3 and

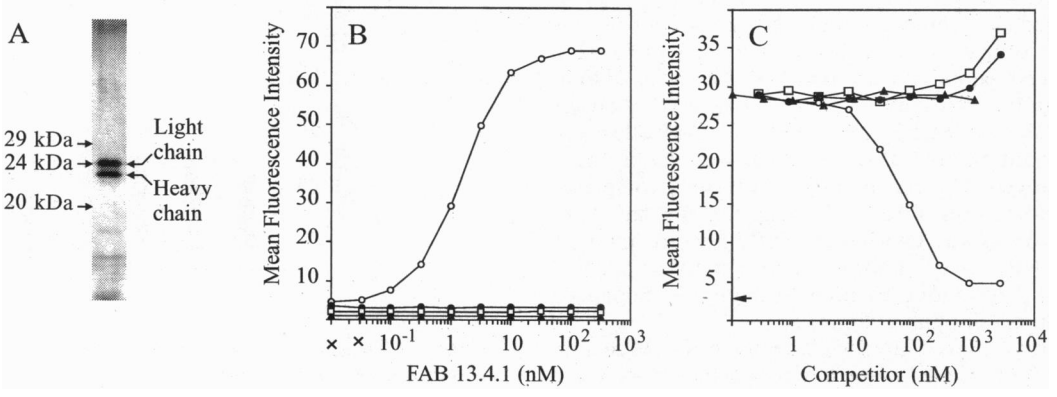

FIG. 2. Purification and binding characteristics of Fab13.4.1. (*A*) Soluble Fab fragments of Fab13.4.1 were prepared and purified as described in *Materials and Methods* and analyzed by SDS/polyacrylamide gel electrophoresis under reducing conditions followed by detection with the silver staining method. (*B*) FACS analysis of binding of purified Fab13.4.1 to latex beads coated with $Ha_{255-262}/K^k$ (○), NP_{50-57}/K^k (●), K^k (□), or $Ha_{255-262}$ (▲). Purified H-2K^b molecules complexed with endogenous peptide were also tested and showed no binding to Fab13.4.1 (results not shown). Coated latex beads were incubated with various concentrations of Fab13.4.1 and analyzed by FACS. (*C*) Competition for binding. Latex beads coated with $Ha_{255-262}/K^k$ were incubated with 3 nM Fab13.4.1 and various concentrations of soluble $Ha_{255-262}/K^k$ complexes (○), NP_{50-57}/K^k complexes (●), K^k (□), or $Ha_{255-262}$ (▲) and then analyzed by FACS.

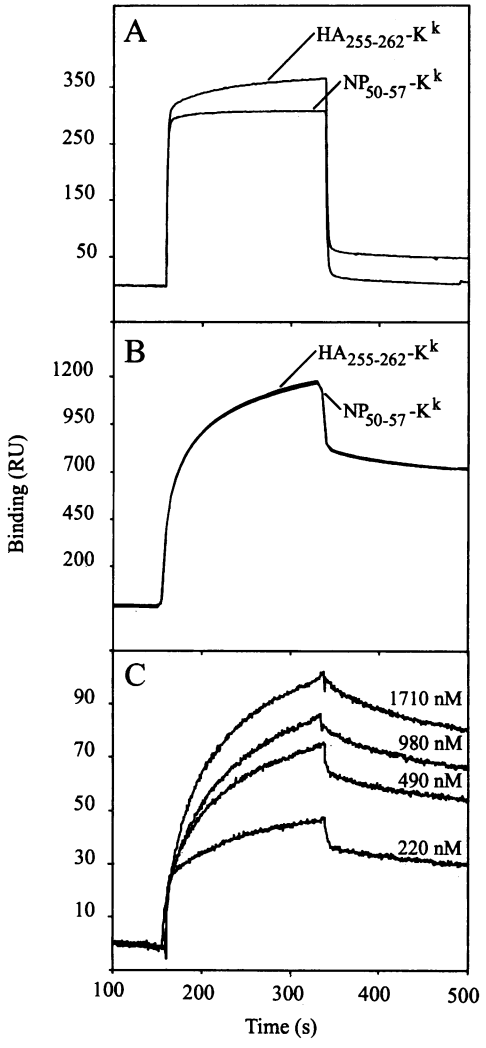

FIG. 3. BIAcore measurements. (*A* and *B*) BIAcore sensorgrams of $HA_{255-262}/K^k$ and NP_{50-57}/K^k complexes binding to immobilized Fab 13.4.1 (*A*) or H100-27R55 (anti-K^k) (*B*). Complexes were diluted to a concentration of 500 nM and passed over the immobilized antibodies for 3 min. (*C*) Kinetic binding curves of $HA_{255-262}/K^k$ binding to Fab 13.4.1. Complexes were applied in various concentrations with a 3-min association phase and a 6-min dissociation phase. The actual concentrations are shown on the sensorgram. All binding curves are expressed as RU as a function of time.

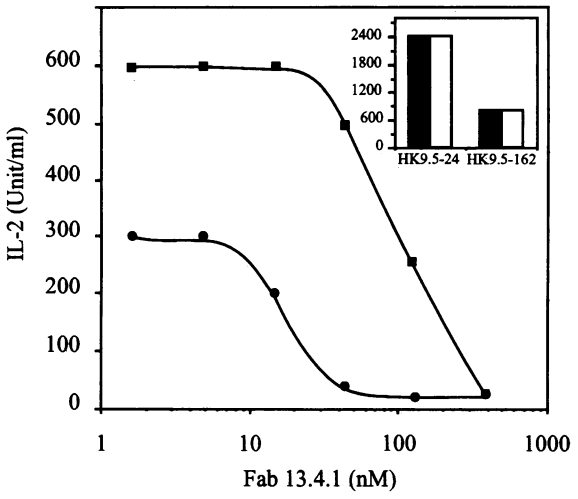

FIG. 4. Specific inhibition of peptide-specific, MHC-restricted T-cell responses. RMA-S·K^k cells were pulsed overnight at 26°C with suboptimal concentrations of $Ha_{255-262}$ or NP_{50-57} and cocultured with the $Ha_{255-262}$-specific T-cell hybridoma lines in the presence of various concentrations of Fab13.4.1. As a control, NP_{50-57}-specific T-cell hybridoma cells were cocultured in the presence (solid bars) or absence (open bars) of the maximal concentration (390 nM) of Fab13.4.1 (*Inset*). The suboptimal concentrations used were 6 mM $Ha_{255-262}$ for presentation to HK8.3-6F8 (●), 0.3 mM $Ha_{255-262}$ for presentation to HK8.3-5H3 (■), 0.3 mM NP_{50-57} for presentation to HK9.5-24, and 3 mM NP_{50-57} for presentation to HK9.5-162.

HK8.3-6F8 (Fig. 4), whereas no effect was observed on two NP_{50-57}-specific, K^k-restricted, T-cell hybridomas, HK9.5-24 and HK9.5-162 (Fig. 4 *Inset*). Hence, Fab13.4.1 recognizes a $Ha_{255-262}/K^k$-dependent epitope which is spatially or allosterically related to the epitope recognized by the T-cell hybridomas HK8.3-5H3 and HK8.3-6F8. The concentration of Fab13.4.1 needed to obtain inhibition of the two $Ha_{255-262}$-specific, K^k-restricted T-cell hybridomas was close to the K_D value measured by the BIAcore analysis.

DISCUSSION

Only a few sporadic publications have reported the generation of self-MHC-restricted antibodies by conventional means (12–14), whereas others have reported that their attempts have failed (15, 16). More recently, a few anti-peptide/MHC antibodies have been reported (17–19). The molecular basis for the past difficulties may be found in the recently solved structures of peptide/MHC class I complexes (20–23). Peptides are deeply buried inside the MHC and are presented as extended mosaics of peptide residues intermingled with self-MHC residues (21–23). Strikingly, no more than 100–300 Å^2 of MHC class I-bound peptides is facing outwards and thus available for direct recognition (21, 23). Self-MHC is thought to account for the majority of the peptide/MHC complex surface presented to T cells (21, 24). Antibodies recognizing protein molecules engage about 800 Å^2 of their ligand (ref. 25; reviewed in ref. 26), so that antibody recognition of a peptide/MHC complex would also have to be dominated by self-MHC. However, antibodies are not selected for being self-MHC-restricted; rather, such antibody specificities might be deleted or silenced (27, 28), explaining why it is so difficult to raise peptide/MHC-specific antibodies. T cells are faced with the same problem of recognizing a ligand dominated by self-MHC, and an entire organ, the thymus, has been devoted to educate T cells. Positive and negative selection processes delete the vast majority of maturing thymocytes and only a small minority enter the circulation as mature nonautoreactive, yet self-MHC-restricted, T cells (29, 30).

Faced with the problem of isolating rare antibody specificities, the selection power of the phage display technology (refs. 31–33; reviewed in ref. 34) is particularly helpful. This technology uses either libraries that have been enriched for the presence of the desired specificity through immunization procedures or libraries of such vast diversity and size that they are likely to contain antibody specificities for any conceivable epitope (35). To determine whether a selected immunization protocol has worked it is important to note that the early immune response is dominated by antibodies generated from germline variable genes with affinities in the micromolar range, while increasing somatic hypermutations result in affinities in the nanomolar range (36). Similarly, phage display antibody fragments isolated from immunization-based libraries in general have 1000-fold higher affinities than those isolated from naive libraries (37–39).

The fact that pSAN13.4.1 described in the present work was isolated from a relatively small library of about 10^7 genes and yet is highly specific, with an affinity in the nanomolar range, strongly indicates that the mice we used for immunization indeed responded to the $Ha_{255-262}/K^k$ complex by generating high-affinity antibodies. Thus, the selected immunization pro-

tocol most likely was an important factor in the generation of Fab 14.3.1.

The observation that only a single anti-$Ha_{255-262}/K^k$ antibody was isolated in the present work may reflect that only one such specificity exists or that other specificities were not generated during the immune response because the immune system will not easily generate and tolerate self-MHC-restricted antibodies. It is possible that the use of large synthetic repertoires with no such restrictions (35) will allow isolation of more variants. Alternatively, weakly binding clones could become lost during the selection procedure itself, resulting in the isolation of a single, strongly binding clone. These issues will be answered as more recombinant peptide/MHC-specific antibodies are produced.

The availability of peptide-specific, MHC-restricted recombinant antibodies may be useful both scientifically and clinically. Such antibodies are, owing to their soluble nature and their high affinity and stability, well suited for detecting the presence of T-cell epitopes under conditions (e.g., immunoprecipitations, immunohistochemistry) which preclude using T cells or recombinant soluble T-cell receptor. Thus, questions relating to how and where certain events occur during antigen presentation may be addressed directly, and the expression of T-cell epitopes on the antigen-presenting cell may be visualized and quantitated. Clinically, antibodies of peptide/MHC specificity might be useful diagnostically since mutations or intracellular infections normally reserved for T-cell recognition would be available to the antibodies, too. Also, new ways of manipulating T-cell responses may be devised. Intracellularly located viruses, bacteria, or parasites are normally presented by MHC class I and recognized by cytotoxic T cells rather than by antibodies. Antibodies that are specific for peptides presented in complex with MHC class I molecules could be conjugated to toxins and used to mimic T-cell responses eradicating, for example virus-infected cells where T cells had failed to do so. Alternatively, anti-peptide/MHC antibodies may block inappropriate immune T-cell responses such as those leading to autoimmunity (19). The generation of blocking antibodies would require knowledge about the disease-inducing T-cell epitope and the restricting MHC molecule, requirements that may be fulfilled in the future (40–42).

We thank Claus Koch and Ron Shymko for help in the BIAcore analysis. This work was supported by the Danish Medical Research Council, the Biotechnology Centers for Signal Peptide Research and Medical Biotechnology, the Danish Cancer Society, the Alfred Benzon Foundation, and the Novo-Nordisk Foundation.

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