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EXPRESSION OF A FUNCTIONAL CHIMERIC Ig-MHC CLASS II PROTEIN[1]

JÖRG ZWIRNER,* WINFRIED WEISSENHORN,† LARS KARLSSON,* ANDREA BECKER,* ERNST P. RIEBER,† GERT RIETHMÜLLER,† ELISABETH H. WEISS,† PER A. PETERSON,* AND GEORG WIDERA[2]*

*From the *Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037, and †Institut für Immunologie, Ludwig-Maximilians-Universität München, 8000 München 2, Federal Republic of Germany*

We have generated a chimeric protein molecule composed of the α- and β-chains of the MHC class II I-E molecule fused to antibody V regions derived from anti-human CD4 mAb MT310. Expression vectors were constructed containing the functional, rearranged gene segments coding for the V region domains of the antibody H and L chains in place of the first domains of the complete structural genes of the I-E α- and β-chains, respectively. Cells transfected with both hybrid genes expressed a stable protein product on the cell surface. The chimeric molecule exhibited the idiotype of the antibody MT310 as shown by binding to the anti-idiotypic mAb 20-46. A protein of the anticipated molecular mass was immunoprecipitated with anti-mouse IgG antiserum. Furthermore, human soluble CD4 did bind to the transfected cell line, demonstrating that the chimeric protein possessed the binding capacity of the original mAb. Thus, the hybrid molecule retained: 1) the properties of a MHC class II protein with regard to correct chain assembly and transport to the cell surface; as well as 2) the Ag binding capacity of the antibody genes used. The generation of hybrid MHC class II molecules with highly specific, non-MHC-restricted binding capacities will be useful for studying MHC class II-mediated effector functions such as selection of the T cell repertoire in thymus of transgenic mice.

Class II (Ia) MHC molecules I-A and I-E are heterodimeric integral membrane proteins composed of noncovalently linked α- (H chain) and β- (L chain) glycoprotein chains. Each chain consists of two extracytoplasmic domains of approximately 100 amino acids, with the membrane proximal domain showing significant homology to Ig constant regions. These are followed by a transmembrane and short intracytoplasmic segment for each α- or β-molecule (1). The expression of Ia Ag in thymus is restricted to epithelial cells, dendritic cells, and macrophage-like cells, which play an important role in the generation of class II MHC restriction and self tolerance of the T cell repertoire (2).

Thymocytes and stromal cells interact via TCR and MHC molecule whose Ag binding groove has been occupied by fragmented Ag (3). However, so-called superantigens like endogenous MLS proteins or exogenously derived staphylococcal enterotoxins skew the T cell repertoire by directly binding to specific β-chains of the TCR of thymocytes (4).

Imitating the capacity of superantigens to directly bind to T cells and modulate the T cell repertoire, we envisaged a hybrid Ig-MHC class II molecule with the V regions of both chains of an antibody joined to the MHC class II α- and β-genes, respectively. These constructs would exhibit three desirable properties. 1) They could bind any desired surface molecule on thymocytes, if the appropriate antibody is chosen. 2) The structure of the membrane proximal protein domains of the MHC class II chains, as well as the transmembrane and cytoplasmic regions of these molecules would not be modified in these hybrid genes. Therefore, the biologic properties of the chimeric MHC class II proteins regarding intracellular signaling would remain unaltered. 3) The Ig-MHC class II molecules could be investigated in immunologically relevant tissues in vivo using the transgenic mouse technique.

A basic prerequisite for these studies is to prove the correct assembly, surface expression, and Ag recognition of such a chimeric molecule.

Chimeric molecules between different members of the Ig superfamily have been shown to be expressed on the cell surface, e.g., chimeras between MHC class I and MHC class II molecules (5), and hybrids between Ig and TCR chains (6). A class of novel proteins, the immunoadhesins, have been generated as chimeric molecules between different members of the Ig superfamily and Ig domains (7).

Here we describe the construction of hybrid genes consisting of the MHC class II E α- and E β-chains and the V regions of the H and L chains of the anti-human CD4 mAb MT310. The resulting hybrid protein is expressed on the cell surface of the transfected cell line A20, is recognized by anti-idiotypic antibody, and binds sCD4[3].

MATERIALS AND METHODS

Generation of Chimeric Genes

V_L and V_H gene segments. Genomic phage clones containing rearranged VJ_L (phage KaWe 3/310) and VDJ_H (phage HCWe 5/310)

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[2] Address correspondence and reprint requests to Dr. Georg Widera, The Scripps Research Institute, Department of Immunology, 10666 North Torrey Pines Road, La Jolla, CA 92037.

[3] Abbreviation used in this paper: sCD4, soluble CD4.

were derived from the murine anti-human CD4 mAb producing hybridoma MT310 (IgG1κ) (W. Weissenhorn, manuscript in preparation). The 2.6-kb *Eco*RI fragment containing promotor sequences, VDJ_H, and the IgH chain enhancer was subcloned into plasmid pSPT19.

I-Eα- and β-gene segments. A 7.5-kb *Kpn*I subclone from the genomic cosmid clone LS3/15 contains exons 3 to 6 of the I-Eβ^b gene. The 2-kb *Eco*RI subclone from this cosmid contains exon 2 (8). The −3.2-kb Eα^d construct contains the structural gene of the I-Eα chain and 3.2 kb of 5′-flanking sequences (9).

Hybrid vectors. To generate the V_H containing chimeric vector, we cloned the 2.6-kb *Eco*RI fragment containing leader and VDJ_H into plasmid −3.2-kb Eα that had been cut with *Nco*I and *Bgl*II removing the promotor region, exon 1 and exon 2 (leader and α1-domain). To enable selection for cell growth in the presence of G418 (GIBCO), the *Cla*I-*Pvu*I fragment of cosmid vector pTCF (10) containing the neomycin resistance gene was introduced into the *Cla*I-*Pvu*I site of the V_H-Eα hybrid gene.

To generate the V_L containing chimeric vector V_L-Eβ, we introduced the 2.5-kb *Hin*dIII-*Eco*RI fragment of KaWe3/310 containing the leader and VJ_L and about 200 bp of 5′-flanking region sequences into the *Sma*I site of the plasmid pBluescript KS. The 7.5-kb *Kpn*I fragment from cosmid clone LS 3/15 (exons 3 to 6 of I-Eβ) was then cloned into the *Hin*dIII site of the vector. The *Hin*dIII-*Sst*I fragment of the CMV enhancer (11) was inserted into the *Not*I site.

Transfection

The mouse B cell lymphoma line A20 was transfected by electroporation using a BTX Transfector 300 (Biotechnologies & Experimental Research Inc.) set at 300 V and 800 μF. After pulsing, cells (1×10^7 in PBS) were plated at 2×10^3 cells/well in 96-well plates. Medium containing G418 (GIBCO) was added after 24 h at a final concentration of 750 μg/ml.

Antibodies

mAb MT310 (mouse IgG1κ) recognizes human CD4 (12). mAb 20–46 (mouse IgG1κ) was raised against mAb MT310 and exhibits anti-idiotypic reactivity. Transfection experiments with different L chains demonstrated that anti-idiotypic mAb 20–46 binds the H chain of mAb MT310 separately. Additional mutational analysis of the V_H region of mAb MT310 showed that mAb 20–46 specifically reacts against the complementary determining region 3 (W. Weissenhorn, manuscript in preparation). mAb 20–46 competes with human CD4 for binding to mAb MT310, which is commercially available as mAb Dako T4 (Dakopatts). mAb MT310 does not block mAb OKT4 (Ortho) from binding to human CD4, demonstrating their reactivity against different epitopes (12).

Flow-Cytometric Analysis

Detection of the hybrid molecules on the cell surface of transfectants was performed by staining cell suspensions with fluorescent reagents and visualizing them on the Becton-Dickinson flow cytometer FACS STAR. Cells (1×10^6) in 100-μl PBS containing 2.5% FCS and 0.05% NaN_3 were incubated with 0.25 μg of anti-idiotypic mAb 20–46 or negative control antibody MOPC 21 (IgG1, Sigma). Second step reagent was fluorescein-conjugated rabbit anti-mouse IgG1 (Cappel). Histograms represent the log of fluorescence intensity vs cell number.

To confirm the Ag specificity of our hybrid molecules, we incubated sCD4 (13) (gift of Biogen Inc.) at 0.2 μg/100 μl in 96-well plates with 5×10^5 transfectants for 30 min at 4°C. After washing, cells were stained with mAb 20–46, OKT4-FITC (mouse IgG2b; Ortho), and DakoT4-FITC (mouse IgG1; Dakopatts) as well as mAb MOPC 141 (mouse IgG2b; Sigma) and MOPC 21 (mouse IgG1; Sigma) as negative control after conjugation to FITC (Sigma).

Immunoprecipitation

Transfectants (2×10^7 cells) were labeled by culturing them for 4 h in the presence of 0.3 mCi [^{35}S]methionine (Amersham). Cell pellets were lysed at 4°C for 30 min in PBS containing 1% Triton X-100 and 2 mM PMSF. Lysates were precleared by incubation with protein A-Sepharose CL-4B (Pharmacia) that had been preabsorbed with rabbit serum. Rabbit anti-mouse Ig (Dakopatts) or mAb 14–4–4S (anti-I-E$\alpha^{k,d}$, IgG2a) was absorbed to protein A-Sepharose and incubated with cell lysates for 4 h at 4°C and then collected by centrifugation. After washing in PBS containing 0.1% Triton X-100, proteins were eluted from protein A-Sepharose, and endoglycosidase H (Boehringer Mannheim) treatment was performed as described (14). One-dimensional SDS-PAGE through 10% gels was performed according to the method of Laemmli (15). Two-dimensional gels were performed as described (16); 7.5 to 12.5% gradient gels were run in the second dimension.

RESULTS

Construction of chimeric genes. Chimeric genes were constructed that encode the V regions of anti-human CD4 mAb MT310 (H and L chains) joined to the MHC class II I-Eα and -β structural genes. For this purpose, genomic clones encoding the I-Eα and -β chains were modified to permit the expression of rearranged Ig VDJ_H and VJ_L exons in place of the first domains of the α- and β-chains of I-E, respectively. A schematic diagram of the resulting chimeric genes is shown in Figure 1 (details are described in *Materials and Methods*). To express these chimeric genes in A20 cells, we preserved intact the Ig regulatory enhancer/promotor sequences of the V_H segment (17). To augment the V_L promotor efficiency, we included the CMV enhancer into the expression vector.

Surface expression of Ig-MHC class II hybrid genes in transfected A20 cells. The V_H-Eα and V_L-Eβ constructs were cotransfected by electroporation into the B cell lymphoma line A20. This cell line is expressing MHC class II proteins and IgG2a. The Ig regulatory elements of our constructs should therefore be suitable for expression in this cell type. After G418 selection, 78 stable clones were obtained. For recognition of the hybrid molecule on the cell surface of transfectants, we used anti-idiotypic mAb 20–46. Flow-cytometric analysis revealed eight positive transfectants. The results for clone V_{310}E15 are shown in Figure 2*e* as compared with negative transfectant V_{310}E3 (Figure 2*a*). All experiments described in this publication were performed with two negative controls: negative transfectant V_{310}E3 and cells of the A20 host cell line. As both gave identical results in all experiments, we document here the results for V_{310}E3 only, showing that the transfection and G418 selection procedure did not alter the host cell line to give unspecific binding of mAb 20–46.

Correct translation and transport of the chimeric polypeptide chains to the cell surface was further analyzed

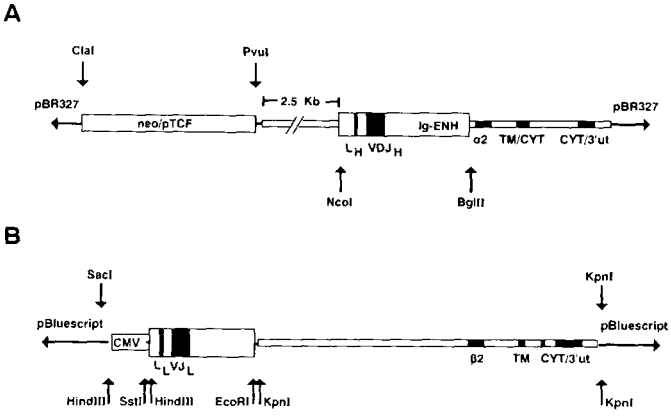

Figure 1. Schematic diagram of the plasmids containing the chimeric V_H-Eα (*A*) and V_L-Eβ (*B*) constructs. Restriction enzymes *above bars* denote available restriction sites; restriction sites lost during subcloning are indicated *below bars*. *Small bars* represent the MHC class II structural gene segments (*black boxes* depict the α2 and β2, transmembrane, and cytoplasmic domains). *Large bars* represent the Ig gene segments of H and L chain (*black boxes* illustrate leader and VDJ/VJ sequences; *open boxes* comprise 5′-flanking and intron sequences plus Ig enhancer in *A*). *Medium-sized bar* represents neomycin resistance gene from cosmid vector pTCF in *A* and CMV enhancer in *B*.

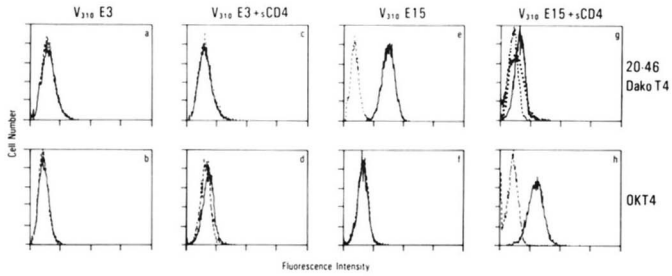

Figure 2. Flow-cytometric analysis of transfectants V_{310}E3 and V_{310}E15, which were stained with mAb 20–46 (——), mAb DakoT4 (· · · ·), or mAb MOPC 21 (– – – –) as negative control, followed by fluorescein-conjugated goat anti-mouse IgG1 (*upper panels*). In the *lower panels*, cells were stained with fluorescein-conjugated mAb OKT4 (——) or MOPC 141 (– – – –) as negative control. Cells in *c*, *d*, *g*, and *h* were incubated with sCD4 before staining.

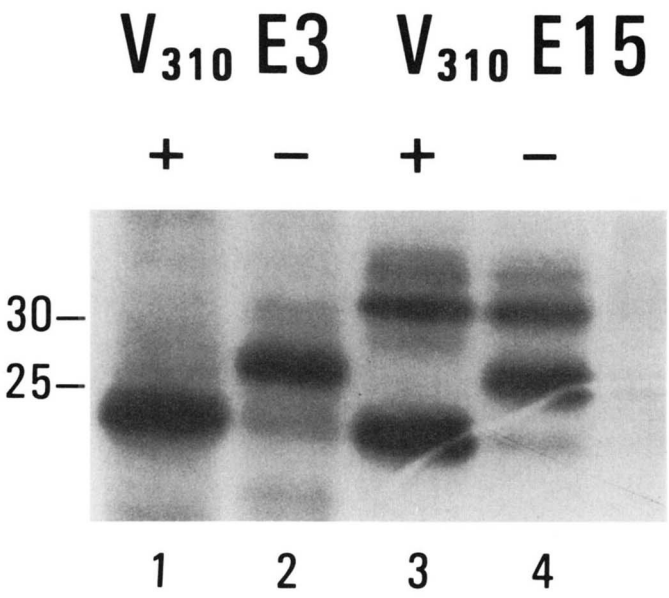

Figure 3. SDS-PAGE on a 10% gel under reducing conditions. Lysates from transfectants V_{310}E3 (*lanes 1* and *2*) and V_{310}E15 (*lanes 3* and *4*) were immunoprecipitated with rabbit anti-mouse Ig. +, Cells treated with endoglycosidase H. Molecular masses are indicated in kDa.

by immunoprecipitation of cell lysates after labeling with [^{35}S]methionine. Rabbit anti-mouse Ig bound to protein A-Sepharose was used as precipitating agent. The results for transfectants V_{310}E3 and V_{310}E15 are presented in Figures 3 and 4.

In contrast to negative transfectant V_{310}E3 (Fig. 3, *lane 2*), the immunoprecipitate of clone V_{310}E15 displayed two bands of about 33 kDa (weak) and 31 kDa (strong), respectively (Fig. 3, *lane 4*). These bands did not change molecular mass after digestion with endoglycosidase H (Fig. 3, *lane 3*), providing evidence that the protein chains had passed through the Golgi apparatus. The bands most likely comprise the α- and β-chains of our hybrid molecule as the α- and β-chains of intact I-E have been shown to migrate in the range of about 30 to 35 kDa (18). The lower bands of about 23 kDa (Fig. 3, *lanes 1* and *3*) and 26 kDa (Fig. 3, *lanes 2* and *4*) most probably represent endoglycosidase H sensitive free intracellular L chain, escaping the preclearing procedure with protein A-Sepharose.

Two-dimensional gel electrophoresis of mAb 20–46 precipitates from surface-expression positive transfectant V_{310}E15 displayed three sets of spots corresponding to the 33-, 31-, and 26-kDa bands in the one-dimensional

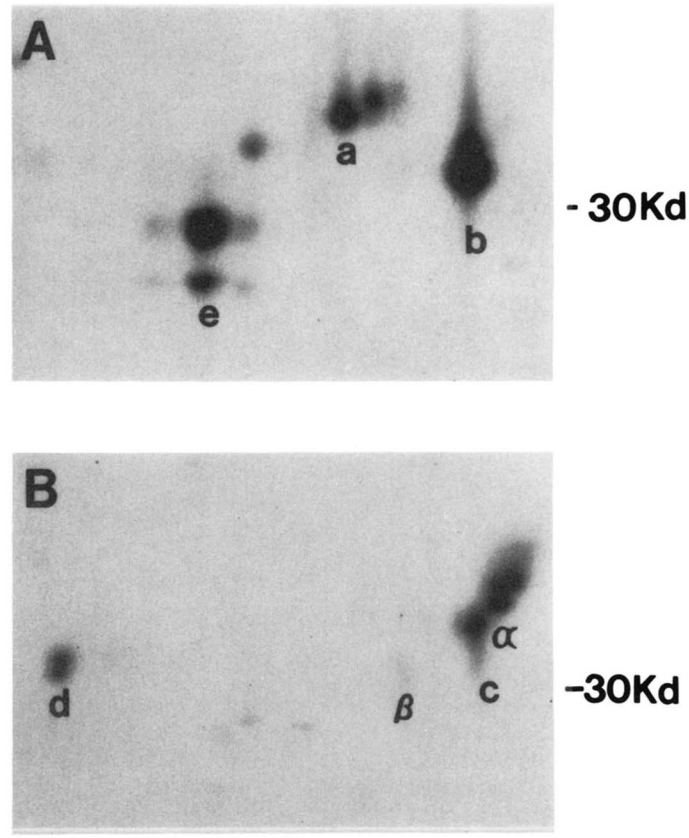

Figure 4. Two-dimensional SDS-PAGE on a 7.5 to 12.5% gradient gel under reducing conditions. Proteins were resolved in nonequilibrium pH gradient gel electrophoresis in the horizontal (basic proteins on the *left*) and by SDS-PAGE in the *vertical dimension*. Lysates from transfectants V_{310}E15 (*A*) and V_{310}E3 (*B*) were immunoprecipitated with rabbit anti-mouse Ig and anti-I-E mAb 14–4–4S, respectively. *Letters* denote position of peptides. *a* and *b*, 33-kDa and 31-kDa hybrid molecule chains; *c*, I-E molecule peptides; α, α-chain; β, β-chain; *d*, invariant chain; *e*, Ig L chain.

analysis in Figure 3, *lane 4*. (Fig. 4*A*, *spots a*, *b*, and *e*). Deducing from the molecular mass, we assume that spots *a* and *b* in Figure 4*A* represent the α- and β-chains of the hybrid Ig-MHC class II molecule, with *spot e* most likely representing internal light chain protein that escaped preclearing.

When immunoprecipitates by anti-I-E mAb 14–4–4S of labeled lysates from clone V_{310}E3 were analyzed by two-dimensional gel electrophoresis, the typical I-E pattern was observed (Fig. 4*B*): I-E protein spots at 33 kDa (α-chain) and 31 kDa (β-chain), and the coprecipitated invariant chain (molecular mass, 31 kDa), located exactly as described by Jones et al. (19). Inasmuch as the labeling was done with [^{35}S]methionine, the I-E β-chain shows up considerably weaker than the α-chain. The appearance of multiple isoelectric spots in the two-dimensional analysis is due to post-translational carbohydrate modification. Comparing the immunoprecipitations of the Ig-MHC class II hybrid molecules by mAb 20–46 with the I-E immunoprecipitations, we conclude that no significant amounts of invariant chain or endogenous I-E protein were coprecipitated together with the hybrid molecules.

Ig-MHC class II hybrid molecules retain Ag binding capacity. To test the antibody specificity of the Ig-MHC class II hybrid molecules, we investigated whether the chimeric molecules on the cell surface have retained the Ag binding capacity of the anti-human CD4 mAb MT310. Transfectants were incubated with sCD4 and subse-

quently stained with mAb 20–46, anti-human CD4 mAb Okt4 or DakoT4. mAb DakoT4 is identical with mAb MT310 and commercially available. OKT4 and DakoT4 recognize different epitopes and do not cross-block each other (12). Soluble CD4 bound specifically to transfectants should: 1) be accessible to mAb OKT4, which recognizes a different epitope on sCD4; 2) block binding of mAb 20–46 to transfectants as its idiotypic determinant on the V region of mAb MT310 is blocked by CD4 (E. P. Rieber, unpublished data); and 3) not be recognized by mAb DakoT4, because the chimeric cell surface protein and mAb DakoT4 use identical V regions thus recognizing identical epitopes on the Ag. Flow-cytometric results as displayed in Figure 2 clearly support these assumptions: *b* and *f* show negative staining for mAb OKT4 being reversed for V_{310}E15 after preincubation with sCD4 (*h*); positive staining of V_{310}E15 with mAb 20–46 (*e*) is greatly reduced after preincubation with sCD4 (*g*). As expected, mAb DakoT4 does not recognize sCD4 bound to V_{310}E15 (*g, dotted line*).

DISCUSSION

In this article, we describe a novel class of MHC class II I-E molecules whose binding capacity has been modified by replacing its N-terminal domain with the variable regions of an antibody. mAb MT310 against human CD4 was chosen as source of the Ig V regions because of the availability of: 1) anti-idiotypic antibodies specifically recognizing mAb MT310 for fast and efficient screening of transfectants; and 2) CD4 in soluble form to demonstrate the Ag specificity of our construct.

We designed MHC class II I-E constructs with the V_H and V_L regions of mAb MT310 in place of the α1 and β1 domains of the α and β chains of I-E, respectively. Positive transfectants were obtained when the chimeric α- and β-chain genes were cotransfected into the mouse B cell lymphoma line A20. There might be several reasons for the fact that only about 10% of the stable G418 resistant clones obtained in our transfection showed surface expression of the idiotype detectable by mAb 20–46. For once, we were using Ig promoters to drive our hybrid constructs, aided by the homologous enhancer (V_H) or by the heterologous CMV enhancer (V_L). These promoter/enhancer pairs should express well in lymphoid cells, but it is conceivable that promoter/enhancer pairs of dedicated expression vectors (20) would express at a better rate. So were transfected Ig genes described to be expressed at a reduced rate relative to hybridomas (21). Inasmuch as the aim of our experiments was to determine whether it would be in principle possible to express the hybrid molecules on the cell surface, and inasmuch as we obtained eight positive clones to test the function of these molecules, we did not further optimize the expression of our constructs.

Surface expression of our chimeric molecules might seem somewhat surprising. Previous studies have documented allele-dependent control of MHC class II cell surface expression. It was found that haplotype-matched α- and β-chains were transported to the membrane with high efficiency, whereas haplotype-mismatched α/β-pairs showed variable expression with most combinations yielding low to intermediate patterns (22, 23). Our results indicate that V_H and V_L regions replacing the α1 and β1 domains of I-Eα and -β, respectively, do not impair the protein transport mechanism to the cell membrane, which is so easily imbalanced in haplotype-mismatched MHC class II chain pairs. Endogenous MHC class II gene products do not seem to interfere with the assembly and expression of our hybrid molecules. In that case, retention of hybrid molecule chains together with endogenous I-Eα and -β chain molecules in the endoplasmic reticulum would be expected, as happens with mismatched α/β-pairs. However, only trace amounts of endogenous I-E protein were immunoprecipitated together with the hybrid molecules. In addition, endoglycosidase H digestion of immunoprecipitates reveals the great majority of hybrid molecules being resistent to cleavage, suggesting its transport to the cell surface (24). We assume that this is also true when V regions of other antibodies are used. Furthermore, no significant amounts of invariant chain were coprecipitated together with the hybrid molecules. Intact MHC class II molecules and invariant chain have been shown to associate very soon after their synthesis in the endoplasmic reticulum (25). This association is thought to regulate the interaction between processed Ag and MHC class II molecules during Ag presentation. The lack of binding of invariant chain to the chimeric MHC class II molecules proves their unique conformational properties as acquired by the antibody V regions.

To maintain the capability for physiologic, MHC class II-like signal transduction by our hybrid molecules, we did not modify the 3′-parts of the structural genes of I-Eα and -β. This might be of importance, inasmuch as it has been shown that truncated α- and β-chains of I-A^k displayed profound defects in intracytoplasmic signaling (26).

Our successful attempts to express chimeric MHC class II molecules that exhibit binding capacity to natural MHC class II ligands in a non-MHC-restricted manner pave the way for an approach to design MHC molecules of any desired specificity, provided that such specificity can be predefined by a mAb. Of particular interest are mAb specific for T cell-associated Ag involved in the selection of the T cell repertoire in vivo like the α/β-TCR, γ/δ-TCR, CD4 and CD8, or mAb with reactivity against specific TCR vβ chains (27, 28). Several of these antibodies have been used in vitro in thymus organ culture experiments, and distinct effects on the T cell repertoire have been documented (29, 30).

In conclusion, we have described the feasibility of a system designed to endow antibody specificity to MHC class II molecules. We have shown the correct assembly, surface expression, and Ag recognition of such chimeric molecules and discussed future applications of the presented system.

REFERENCES

1. **Kaufman, J. F., C. Affray, A. J. Korman, D. A. Shackelford, and J. Strominger.** 1984. The class II molecules of the human and murine major histocompatibility complex. *Cell 36:1.*
2. **Sprent, J., and S. R. Webb.** 1987. Function and specificity of T cell subsets in the mouse. *Adv. Immunol. 41:39.*
3. **Schwartz, R. H.** 1986. Immune response (Ir) genes of the murine major histocompatibility complex. *Adv. Immunol. 38:31.*
4. **Marrack, P., and J. Kappler.** 1990. The staphylococcal enterotoxins and their relatives. *Science (Washington DC) 248:705.*
5. **Golding, H., J. McCluskey, T. I. Munitz, R. N. Germain, D. H. Margulies, and A. Singer.** 1985. T-cell recognition of a chimeric class II/class I MHC molecule and the role of L3T4. *Nature (Lond.) 317:425.*
6. **Goverman, J., S. M. Gomez, K. D. Segesman, T. Hunkapiller, W. E. Laug, and L. Hood.** 1990. Chimeric immunoglobulin-T cell receptor

proteins form functional receptors: implications for T cell receptor complex formation and activation. *Cell 60:929.*

7. **Capon, D. J., S. M. Chamow, J. Mordenti, S. A. Marsters, T. Gregory, H. Mitsuya, R. A. Byrn, C. Lucas, F. M. Wurm, J. E. Groopman, S. Broder, and D. H. Smith.** 1989. Designing CD4 immunoadhesins for AIDS therapy. *Nature (Lond.) 337:525.*
8. **Widera, G., and R. A. Flavell.** 1984. The nucleotide sequence of the murine I-Eβ^b immune response gene: evidence for gene conversion events in class II genes of the major histocompatibility complex. *EMBO J. 3:1221.*
9. **Widera, G., L. C. Burkly, C. A. Pinkert, E. C. Bottger, C. Cowing, R. Palmiter, R. L. Brinster, and R. A. Flavell.** 1987. Transgenic mice selectively lacking MHC class II (I-E) antigen expression on B cells: an in vivo approach to investigate Ia gene function. *Cell 51:175.*
10. **Grosveld, F. G., T. Lund, E. J. Murray, A. L. Mellor, H. H. M. Dahl, and R. A. Flavell.** 1982. The construction of cosmid libraries which can be used to transform eucaryotic cells. *Nucleic Acids Res. 10:6715.*
11. **Boshart, M., F. Weber, G. Jahn, K. Dorsch-Habler, B. Fleckenstein, and W. Schaffner.** 1985. A very strong enhancer is located upstream of an immediate early gene of the cytomegalovirus. *Cell 41:521.*
12. **Sattentau, Q. J., A. G. Dalgleish, P. C. Weiss, and P. C. L. Beverley.** 1986. Epitopes of the CD4 antigen and HIV infection. *Science (Washington DC) 234:1120.*
13. **Fisher, R. A., J. M. Bertonis, W. Meier, V. A. Johnson, D. S. Costopoulos, T. Liu, R. Tizard, B. Walker, M. S. Hirsch, R. T. Schooley, and R. A. Flavell.** 1988. HIV infection is blocked in vitro by recombinant soluble CD4. *Nature (Lond.) 331:76.*
14. **Sege, K., L. Rask, and P. A. Peterson.** 1981. Role of beta$_2$-microglobulin in the intracellular processing of the HLA antigens. *Biochemistry 20:4523.*
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.) 227:680.*
16. **Jones, P. P.** 1980. Analysis of radiolabeled lymphocyte proteins by one- and two-dimensional gel electrophoresis. In *Selected Methods in Cellular Immunology.* B. P. Mishell and S. P. Shigii, eds. Freeman, San Francisco, p. 398.
17. **Storb, U.** 1987. Transgenic mice with immunoglobulin genes. *Ann. Rev. Immunol. 5:151.*
18. **Lawrance, S. K., L. Karlsson, J. Price, V. Guaranta, Y. Ron, J. Sprent, and P. A. Peterson.** 1989. Transgenic HLA DRα faithfully reconstitutes IE-controlled immune functions and induces cross-tolerance to Eα in Eα^o mutant mice. *Cell 58:583.*
19. **Jones, P. P., D. B. Murphy, D. Hewgill, and H. O. McDevitt.** 1978. Detection of a common polypeptide chain in I-A and I-E subregion immunoprecipitates. *Immunochemistry 16:51.*
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
21. **Dorai, H., and G. P. Moore.** 1987. The effect of dihydrofolate reductase-mediated gene amplification on the expression of transfected immunoglobulin genes. *J. Immunol. 139:4232.*
22. **Braunstein, N. S., and R. N. Germain.** 1987. Allele-specific control of Ia molecule surface expression and conformation: implications for a general model of Ia structure-function relationships. *Proc. Natl. Acad. Sci. USA 84:2921.*
23. **Germain, R. N., D. M. Bentley, and H. Guill.** 1985. Influence of allelic polymorphism on the assembly and surface expression of class II MHC (Ia) molecules. *Cell 43:233.*
24. **Kornfeld, R., and S. Kornfeld.** 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem. 54:631.*
25. **Kvist, S., K. Wiman, L. Claesson, P. A. Peterson, and B. Dobberstein.** 1982. Membrane insertion and oligomeric assembly of HLA-DR histocompatibility antigens. *Cell 29:61.*
26. **Nabavi, N., Z. Ghogawala, A. Myer, J. Griffith, W. F. Wade, Z. Z. Chen, D. J. McKean, and L. H. Glimcher.** 1989. Antigen presentation abrogated in cells expressing truncated Ia molecules. *J. Immunol. 142:1444.*
27. **Kappler, J., N. Roehm, and P. Marrack.** 1987. T cell tolerance by clonal elimination in the thymus. *Cell 49:273.*
28. **Staerz, U., H. G. Rammensee, J. Benedetto, and M. Bevan.** 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on the T cell antigen receptor. *J. Immunol. 134:3994.*
29. **Born, W., M. McDuffie, N. Roehm, E. Kishnir, J. White, D. Thorpe, J. Stefano, J. Kappler, and P. Marrack.** 1987. Expression and role of the T cell receptor in early thymocyte differentiation in vitro. *J. Immunol. 138:999.*
30. **Zepp, F., and U. D. Staerz.** 1988. Thymic selection process induced by hybrid antibodies. *Nature (Lond.) 336:473.*