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 idiotypic specificity 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 (α) 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 I 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).
were derived from the murine anti-human CD4 mAb producing hybridoma MT310 (IgG1s) (W. Weissenhorn, manuscript in preparation). The 2.6-kb EcoRI fragment containing promoter sequences, VDJH, and the IgH chain enhancer was subcloned into plasmid pSP719.

\textbf{I-Eα- and β-gene segments.} A 7.5-kb KpnI subclone from the genomic cosmid clone LS3/15 contains exons 3 to 6 of the I-Eα gene. The 2.6-kb EcoRI subclone from this cosmid contains exon 2 (8).

To generate the \textit{V\textsubscript{H}}, containing chimeric vector \textit{V\textsubscript{H}-E\textsubscript{β}}, we introduced the 2.5-kb HindIII-EcoRI fragment of KaWe3/310 containing the lead and VDJ, and about 200 bp of 5'-flanking region sequences into the SmaI site of the plasmid pBluescript KS. The 7.5-kb KpnI fragment from cosmid clone LS 3/15 (exons 3 to 6 of I-E\textsubscript{α}) was then cloned into the HindIII site of the vector. The HindIII-SstI fragment of the CMV enhancer (11) was inserted into the NotI site.

\textbf{Transfection.} The mouse B cell lymphoma line A20 was transfected by electroporation using a BTX Transfactor 300 (Biotechnological and Experimental Research Inc.) set at 300 V and 800 μF. After pulsing, cells \((1 \times 10^7\text{ cells} / \text{100 μl PBS})\) were plated at 2 \times 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.

\textbf{Antibodies.} mAb MT310 (mouse IgG1s) recognizes human CD4 (12), mAb 20-46 (mouse IgG1) 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 \textit{V\textsubscript{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 mAb MT30 (H and L chains) joined to the MHC class I1 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 transfected cells, we used anti-idiotypic mAb 20-46. Flow-cytometric analysis revealed eight positive transfected. The results for clone V\textsubscript{310},E\textsubscript{15} are shown in Figure 2a as compared with negative transfectant V\textsubscript{310},E\textsubscript{3} (Figure 2a). All experiments described in this publication were performed with two negative controls: negative transfectant V\textsubscript{310},E\textsubscript{3} and cells of the A20 host cell line. As both gave identical results in all experiments, we document here the results for V\textsubscript{310},E\textsubscript{3} only, showing that the transfection and G418 selection procedure did not alter the host cell line to give unspecific binding of mAb 20-46.

\textbf{Correct translation and transport of the chimeric polypeptide chains to the cell surface} was further analyzed 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.

\textbf{RESULTS}

\textbf{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 \(\beta\)-structural genes. For this purpose, genomic clones encoding the I-Eα and \(\beta\) chains were modified to permit the expression of rearranged Ig VDJH and VJL exons in place of the first domains of the \(\alpha\) and \(\beta\)-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/promoter sequences of the \(V\textsubscript{H}\) segment (17). To augment the V\textsubscript{L} promoter efficiency, we included the CMV enhancer into the expression vector.

\textbf{Surface expression of Ig-MHC class II hybrid genes in transfected A20 cells.} The V\textsubscript{310},E\textsubscript{α} and V\textsubscript{310},E\textsubscript{β} 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 surface of transfected cells, we used anti-idiotypic mAb 20-46. Flow-cytometric analysis revealed eight positive transfected. The results for clone V\textsubscript{310},E\textsubscript{15} are shown in Figure 2a as compared with negative transfectant V\textsubscript{310},E\textsubscript{3} (Figure 2a). All experiments described in this publication were performed with two negative controls: negative transfectant V\textsubscript{310},E\textsubscript{3} and cells of the A20 host cell line. As both gave identical results in all experiments, we document here the results for V\textsubscript{310},E\textsubscript{3} only, showing that the transfection and G418 selection procedure did not alter the host cell line to give unspecific binding of mAb 20-46.
Flow-cytometric analysis of transfectants V310E3 and V310E15, 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.

SDS-PAGE on a 10% gel under reducing conditions. Lysates from transfectants V310E3 (lanes 1 and 2) and V310E15 (lanes 3 and 4) were immunoprecipitated with rabbit anti-mouse IgG. Cells treated with endoglycosidase H. Molecular masses are indicated in kDa.

In contrast to negative transfectant V310E3 (Fig. 3, lane 2), the immunoprecipitate of clone V310E15 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 V310E15 displayed three sets of spots corresponding to the 33-, 31-, and 26-kDa bands in the one-dimensional analysis in Figure 3, lane 4. (Fig. 4A, spots a, b, and e). Deducing from the molecular mass, we assume that spots a and b in Figure 4A 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 14-4-48 of labeled lysates from clone V310E3 were analyzed by two-dimensional gel electrophoresis, the typical I-E pattern was observed (Fig. 4B): 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 [35S]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-
quent staining 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 transfec-
tants should: 1) be accessible to mAb OKT4, which rec-
ognizes a different epitope on sCD4; 2) block binding of mAb 20-46 to transfec-
tants yielding low to intermediate patterns (22, 23). Our mAbs recognize different epitopes and do not cross-block each other: 1) MT310 and commercially available. OKT4 and DakoT4 recognize a different epitope on sCD4; 2) block binding of mAb 20-46 to transfectants as its idiotypic determinant (h); positive staining of V310E15 with mAb 20-46 being reversed for V310E15 after preincubation with sCD4 (g). As expected, mAb DakoT4 does not recognize sCD4 bound to V310E15 (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 transfec-
tants; and 2) CD4 in soluble form to demonstratethe Ag specificity of our construct.

We designed MHC class II I-E constructs with the Vn and V regions of mAb MT310 in place of the α1 and β1 domains of the α and β chains of I-E, respectively. Positive transfec-
tants 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 (Vn) or by the heterologous CMV enhancer (V). 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 ex-
pressed 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 docu-
mented allele-dependent control of MHC class II cell sur-
f ace 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 combina-
tions yielding low to intermediate patterns (22, 23). Our results indicate that Vn and V 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 hy-
brit molecules. In addition, endoglycosidase H digestion of immunoprecipitates reveals the great majority of hy-
brit molecules being resistant 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 expressed 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 c/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**