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B-lymphoma cells process and present their endogenous immunoglobulin to major histocompatibility complex-restricted T cells

(antigen-presenting cells/idiotype/MOPC315)

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ABSTRACT **Antigen-presenting B-lymphoma cells were transfected with the gene encoding the immunoglobulin λ2 light chain of MOPC315 cells ($\lambda2^{315}$). The λ2 chain is expressed on the cell surface of the transfectants together with the endogenous heavy chain. The transfectants present an idiotope of the $\lambda2^{315}$ light chain to class II-restricted T-cell clones. Recognition by the T cells requires processing of the $\lambda2^{315}$ light chain. From these data we conclude that B-lymphoma cells constitutively process and present their immunoglobulins. Secretion and reuptake of the light chain was not necessary for the presentation. Thus, B cells bear two types of idiotypes on their membrane, a native form as surface immunoglobulin and a processed form in the context of products of the major histocompatibility complex.**

B cells use immunoglobulin on their cell surface as receptors for native antigen. Every immunoglobulin molecule bears a collection of antigenic determinants, the so-called idiotype, which is localized in the variable region of the molecule. Idiotypes and anti-idiotypic antibodies were postulated by Jerne to form a regulatory network in the immune system (1). This network most likely includes also T cells, since T cells are essential for clonal expansion of B cells.

In contrast to B cells, T cells can recognize only processed forms of antigen in association with products of the major histocompatibility complex (MHC) on the surface of antigen-presenting cells (APCs). Some B cells can function as APCs (2). In 1981 Jørgensen *et al.* (3) proposed that B cells process their surface immunoglobulin and present it in context of their MHC molecules (3). This proposal was based on the findings that T cells specific for an idiotype on the λ2 light chain from MOPC315 cells ($\lambda2^{315}$) recognized denatured forms of $\lambda2^{315}$ (4) and that this recognition was under H-2-linked immune response (Ir) gene control (5). The hypothesis has been supported and extended by later work (6–8). Similar ideas have recently been proposed by others (9, 10), and MHC-restricted T cells specific for idiotypes (11, 12) and allotypes (13) have been described. However, it is still unclear whether MHC-restricted T cells recognize processed forms of immunoglobulin exclusively and whether B cells can process their own immunoglobulin.

To test this directly we have used recently established T-cell clones specific for an idiotope of $\lambda2^{315}$ in the context of the I-E^d MHC antigen (7, 8). These clones, like the T cells studied *in vivo* before (4, 5), recognize an idiotypic determinant on $\lambda2^{315}$ around amino acid positions 94, 95, and 96 (7, 8). At these three positions the λ2 light chain of myeloma protein M315 differs from the germ-line-encoded λ2 sequence due to somatic mutations (14, 15). For this reason, B cells bearing the $\lambda2^{315}$ idiotope should be exceedingly rare in normal BALB/c mice. To obtain a clonal population of idiotope-expressing B cells we have transfected antigen-presenting B-lymphoma cells with the λ2 gene from MOPC315. Here we show that the transfectants express the $\lambda2^{315}$ light chain together with the endogenous heavy chain on the cell surface. At the same time they present a processed form of the idiotope of $\lambda2^{315}$ to the I-E^d-restricted T cells.

MATERIALS AND METHODS

Proteins. $\lambda2^{315}$ light chain of BALB/c myeloma protein M315 (α, λ2) was purified as described (16). FRN, YRN, FSN, and FRT synthetic peptides were kindly provided by K. Hannestad (Institute for Medical Biology, Tromsø, Norway) and J.-P. Briand (Institut de Biologie Moleculaire et Cellulaire, Strasbourg, France). The FRN peptide represents the $\lambda2^{315}$ amino acid sequence from positions 91 to 108. YRN, FSN, and FRT peptides are identical to FRN except for the amino acid exchanges indicated by the one-letter code in position 94, 95, or 96, respectively (6).

Antibodies. K24.199 (anti-I-$A^{d,f,j,v}$) (17) and 13/4 (anti-I-E^d) (18) IgG2a monoclonal antibodies were purified from ascites fluid on staphylococcal protein A-Sepharose. Fluorescein-conjugated monoclonal anti-D3.137 (anti-I-A^d) (19) was kindly donated by M. H. Julius (Basel Institute for Immunology).

Cell Lines. The $CD4^+$ $CD8^-$ T-cell clones specific for the idiotope of $\lambda2^{315}$ and restricted to I-E^d express an αβ heterodimeric receptor and have been described extensively (7, 8). They recognize the FRN peptide (see above), thus localizing the idiotypic determinant of $\lambda2^{315}$ to amino acid positions between 91 and 108 (8). The B-lymphoma cell lines A20J, A20/10, and A20/46 (20) have been obtained from T. Leanderson and M. H. Julius. They were originally thought to be independent, but Southern blot analysis of their immunoglobulin genes suggested that they all might have originated from A20 (data not shown), and two of them (originally called L10 and K46) were therefore renamed A20/10 and A20/46.

Gene Construction and Transfection. The plasmid pSV2λ2 was kindly provided by G. E. Wu (Basel Institute for Immunology). It consists of the 6.6-kilobase (kb) *Eco*RI fragment containing the λ2 gene of MOPC315 (15) inserted into the *Eco*RI site of the expression vector pSV2neo (21). The mouse immunoglobulin heavy chain enhancer (22, 23) contained in a 1-kb *Xba* I fragment of the Sp6 heavy chain gene (kindly provided by A. Traunecker, Basel Institute for Immunology) was inserted into the unique *Xba* I site in the major intron of the λ2 gene, as shown in Fig. 1*a*. This construct was

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Abbreviations: APC, antigen-presenting cell; MHC, major histocompatibility complex.

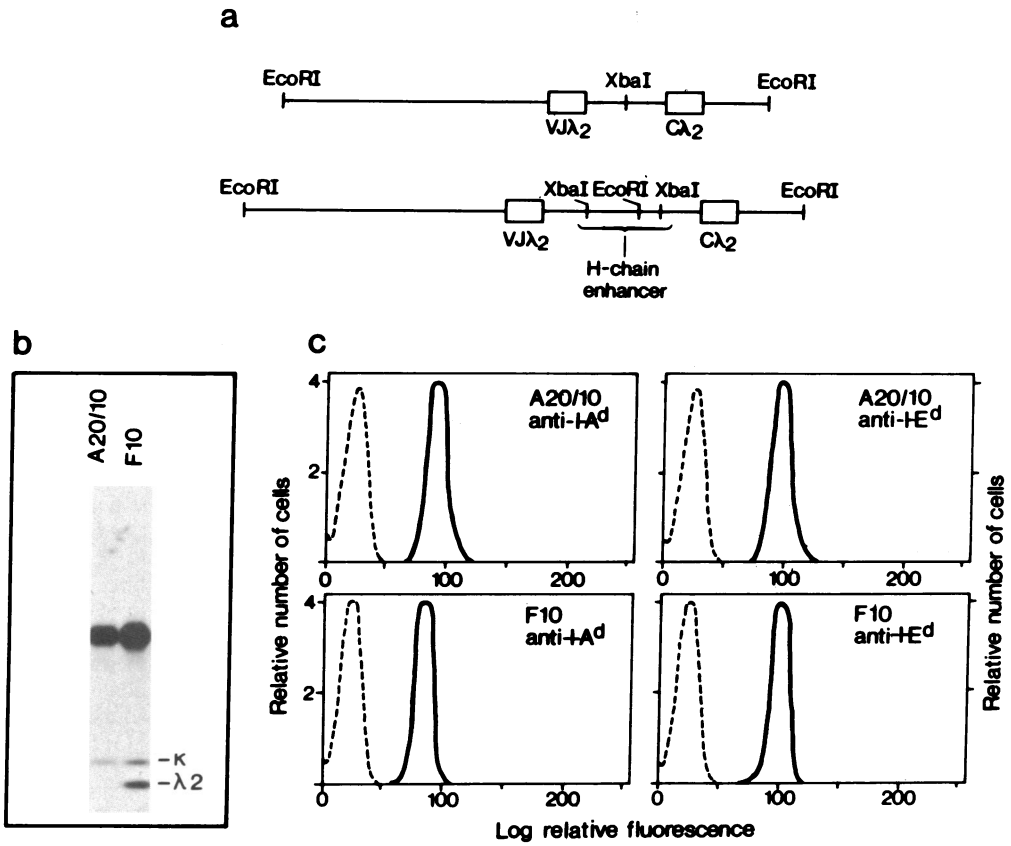

FIG. 1. (*a*) Construct of the λ2 gene of MOPC315 containing the heavy chain enhancer. The 1-kb *Xba* I fragment of the enhancer was inserted into the λ2 gene in the same orientation as it is found in the heavy chain. The modified λ2 gene is integrated in the *Eco*RI site of the expression vector pSV2neo in the opposite transcriptional orientation with respect to the *neo* gene. (*b*) Electrophoretic analysis of ^{125}I-labeled surface proteins of A20/10 and the corresponding transfectant F10 precipitated with anti-mouse immunoglobulin and protein A-Sepharose. Similar results were obtained by biosynthetic labeling with [^{35}S]methionine and binding of anti-κ and anti-λ antibodies to viable cells followed by lysis and immunoprecipitation (data not shown). (*c*) Cell surface analysis of A20/10 and the corresponding transfectant F10 clone in a fluorescence-activated cell sorter using fluorescein-conjugated monoclonal anti-I-A^d D3.137 (kindly provided by M. H. Julius) and anti-I-E^d 13/4 antibodies. λ2 chain, I-A^d, and I-E^d expression was similar for the other cell lines.

transfected into the B-lymphoma cells via protoplast fusion (24). Neomycin-resistant F9, F10, and F11 clones were obtained from A20/46, A20/10, and A20J, respectively.

Fluorescence-Activated Cell Sorter (FACS) Analysis. B-lymphoma cells and transfectants were stained with fluorescein-conjugated anti-I-E^d (13/4) and anti-I-A^d (D3.137) monoclonal antibodies for 30 min on ice, counterstained with propidium iodide, and analyzed on a Becton Dickinson FACS 440 (analysis kindly performed by D. Thorpe).

Immunoprecipitation. Cells were surface iodinated by using glucose oxidase and lactoperoxidase, lysed with 0.5% Nonidet P-40, and, after removal of nuclei, stored at −70°C. Portions were precipitated with rabbit antiserum to mouse immunoglobulin and protein A-Sepharose or with protein A-Sepharose alone. Immunoprecipitates were analyzed on sodium dodecyl sulfate/10% polyacrylamide gels under reducing conditions.

Radioimmunoassay. Two assays were employed. The first detects free λ2^{315} light chains and λ2^{315} light chains complexed to heavy chain equally well. The binding of affinity-purified rabbit anti-λ2^{315} to ^{125}I-labeled free λ2^{315} was inhibited by various amounts of culture supernatant. The complexes were precipitated with goat antiserum to rabbit immunoglobulin as second antibody. Since native M315 was used as standard, only a third of which is λ2 light chain, the given numbers have to be divided by 3 to obtain the concentration of free λ2 light chain (see Fig. 3). The second assay detects free λ2^{315} only (16). Binding of BALB/c anti-λ2^{315} antibodies to iodinated free λ2^{315} was inhibited with various amounts of supernatants. Rabbit antiserum to mouse immunoglobulin was used as second antibody and free λ2^{315} as standard.

Proliferation Assay. B-lymphoma cells or transfectants were treated with mitomycin C (7) or fixed with glutaraldehyde (25) as described. In assays, 3 × 10^4 B-lymphoma cells and 4 × 10^4 T cells (taken 12 days after the last stimulation with antigen) were cultured with or without addition of antigen in flat-bottomed (mitomycin-C-treated APCs) or round bottomed (fixed APCs) microtiter wells for 48 hr before a 12-hr pulse with 1 μCi (1 Ci = 37 GBq) of [*methyl*-^{3}H]thymidine. For inhibitions, monoclonal anti-Ia antibodies were added to APCs 15 min prior to addition of T cells and antigen. Culture conditions have been described (7).

Growth Inhibition Assay. It has previously been shown that the cell clones display λ2^{315}-specific and I-E^d-restricted cytotoxicity (7). The assay was performed essentially as described (7): 5 × 10^3 B-lymphoma cells and 1 × 10^4 cloned T cells irradiated with 2000 rad (1 rad = 0.01 gray) were cultured for 48 hr before a 10- to 18-hr pulse with 1 μCi of [*methyl*-^{3}H]thymidine. Percent inhibition of growth was calculated as [(cpm lymphoma cells alone − cpm lymphoma cells with T cells) ÷ cpm lymphoma cells alone] × 100.

Diffusion Chamber System. Prior to the growth inhibition assay of B-lymphoma cells, parental cells and transfectants were cocultivated in Costar Transwell diffusion chambers (Cambridge, MA) with 3.0-μm pore diameter for various lengths of time. During cocultivation, transfectant F9 or F10 cells were grown in the inside wells and the corresponding

Table 1. Capacity of B-lymphoma cells to present exogenously added immunoglobulin or peptides

Treatment of B-lymphoma cells	Antigen	Antigen conc., μg/ml	[^{3}H]Thymidine incorporated, cpm			
			A20/46		A20/10	A20J
Mitomycin C	None	—	3,238 ± 161		1,497 ± 27	710 ± 161
	λ2^{315}	10	26,020 ± 5481		17,640 ± 3824	15,920 ± 10,620
	λ2^{315}	1	5,974 ± 390		1,241 ± 313	644 ± 31
			Exp. 1	Exp. 2		
Glutaraldehyde	None	—	288 ± 82	285 ± 39	288 ± 82	322 ± 16
	λ2^{315}	100		2,680 ± 468		
	λ2^{315}	10	393 ± 64	1,132 ± 128	227 ± 24	255 ± 27
	FRN	50		13,130 ± 3413		
	FRN	5*		14,160 ± 1159		
	YRN	50		427 ± 56		
	FSN	50		349 ± 20		
	FRT	50		182 ± 131		

The indicated B-lymphoma cells were treated with mitomycin C or glutaraldehyde, incubated with antigen, and then added to the idiotope-specific 4B7 T-cell clone. Incorporation of [*methyl*-^{3}H]thymidine was measured; results are mean ± SD for triplicates.
*Glutaraldehyde-fixed or mitomycin C-treated A20/46 cells presented the FRN peptide at 5 μg/ml but not at 0.8 μg/ml.

parental A20/46 or A20/10 cells were grown in the outside wells. Diffusion of iodinated M315 or λ2^{315} across the membrane reached equilibrium within 2–4 hr (data not shown). Quantification of λ2^{315}-containing molecules in supernatants from both compartments at the time of cell harvest (using complete M315 as standard) showed that cells in the inside and the outside wells were exposed to equal concentrations of λ2^{315}. Identical sets of precultures were harvested at day 4 and day 5 to ensure that day 5 cocultivated parental cells were exposed to higher concentrations of λ2^{315} than day 4 cocultured antigenic transfectants. The day 10 and 11 experiments involved washing and dilution of cells at day 8. The concentrations of cells grown in the two compartments were approximately equal. No transfectants passed through the micropore filter, since cells from the outside wells remained completely sensitive to neomycin.

RESULTS

B-Lymphoma Cells Present a Processed Form of Exogenously Added λ2^{315}. To test whether the B-lymphoma cell lines A20J, A20/10, and A20/46 chosen for transfection were able to present soluble λ2^{315}, the cells were incubated with free λ2^{315} light chain. Table 1 demonstrates that the mitomycin C-treated B-lymphoma cell lines incubated with λ2^{315} at 10 μg/ml but not at 1 μg/ml presented the idiotope to the idiotope-specific 4B7 T-cell clone. Glutaraldehyde fixation of B-lymphoma cells drastically reduced their capacity to present soluble λ2^{315} chain (Table 1). However, fixed or mitomycin C-treated B-lymphoma cells still were equally efficient in presenting a synthetic peptide representing amino acids 91–108 of the λ2^{315} sequence. Peptides in which residues 94, 95, or 96 were exchanged with the germ-line-encoded amino acids were not presented (Table 1). This suggests that idiotope-specific T cells recognize a processed form of the idiotope, in analogy to what has been found for other protein antigens (25).

B-Lymphoma Cells Constitutively Process and Present Endogenously Produced λ2^{315}. To obtain an endogenous source of idiotope the λ2^{315} gene containing the heavy chain enhancer was transfected into the B-lymphoma cells. (As found out later, the enhancer was not essential for the experiments, but it optimized the system.) Neomycin-resistant clones F9, F10, and F11 were obtained from A20/46, A20/10, and A20J, respectively. Confirming previous results on A20 lymphoma cells (20), parental and transfected cells could not be stained with various fluoresceinated antibodies to mouse immunoglobulin, presumably because too little surface immunoglobulin is expressed. However, the λ2^{315} chain together with the parental κ and heavy chain could be precipitated from lysates of surface-iodinated transfectants in a sensitive immunoprecipitation assay (Fig. 1*b* and data not shown). The transfectants secrete small amounts of mainly free λ2^{315} (not complexed to the endogenous heavy chain) as measured by the two different radioimmunoassays. This is significant because on a molar basis free λ2^{315} is 100- to 1000-fold more immunogenic to T cells than M315 is (7). However, super-

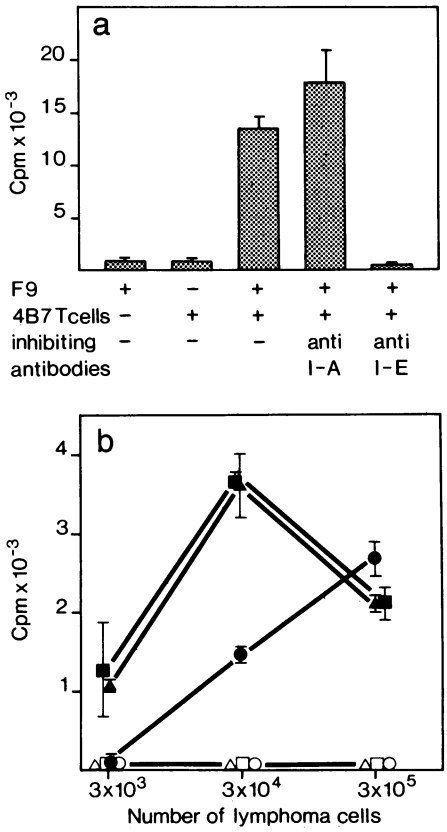

FIG. 2. Ability of transfected B-lymphoma cells to present endogenous idiotope to I-E^d-restricted T cells. (*a*) Proliferative responses of idiotope-specific 4B7 T cells to mitomycin C-treated F9 transfectants. For inhibition, monoclonal antibodies K24.199 (anti-IA^{d,f,j,v}) and 13/4 (anti-I-E^d) were used at 50 μg/ml. (*b*) Proliferative responses of 4B7 T cells to glutaraldehyde-fixed APCs. □, A20/46 + 4B7 T cells; ■, F9 + 4B7; △, A20/10 + 4B7; ▲, F10 + 4B7; ○, A20J + 4B7; and ●, F11 + 4B7. Fixed lymphoma cells or 4B7 T cells alone incorporated <300 cpm of [^{3}H]thymidine.

natants, even of very dense cultures, never contained more than 150 ng of free $\lambda 2^{315}$ per ml. The transfected lymphoma cells expressed I-A^d and I-E^d molecules on the cell surface (Fig. 1*c* and data not shown).

We then investigated whether the $\lambda 2^{315}$ transfectants could induce proliferation of idiotope-specific T cells without the addition of exogenous antigen. As shown in Fig. 2*a*, mitomycin C-treated F9 cells stimulated 4B7 T cells, while the corresponding parental cells A20/46 did not (Table 1). As expected, the proliferation could be inhibited by anti-I-E^d and not by anti-I-A^d antibodies (Fig. 2*a*). Also, glutaraldehyde-fixed transfectants induced proliferation of the 4B7 T-cell clone, while the corresponding parental cell lines did not (Fig. 2*b*). These results indicate that the transfectants continuously process their endogenously produced $\lambda 2^{315}$ polypeptide chain.

The Processed Form of Idiotope Is Not Derived from Secreted $\lambda 2^{315}$. The findings could be explained by reuptake of secreted free $\lambda 2^{315}$. We find this possibility unlikely because the maximum concentration of free $\lambda 2^{315}$ in supernatants of transfectants (150 ng/ml) is much less than that required for exogenously added antigen (>1 μg/ml) in proliferation (Table 1) and growth inhibition (Fig. 3) assays. Even in a long-term pulse experiment, with free $\lambda 2^{315}$ for 5–6 days, it was necessary to add more than 1 μg/ml during pulsing and assay to render parental cells antigenic in proliferation and growth-inhibition assays (data not shown).

Finally, long-term exposure of parental cells to supernatants of transfectants by coculturing parental and transfected cells in a diffusion chamber system did not render the parental B-lymphoma cells antigenic to idiotope-specific T cells as measured by growth-inhibition assay (Fig. 3) and proliferation assay (data not shown). Therefore, the processed $\lambda 2^{315}$ must originate from a source local to the transfected cell.

DISCUSSION

The way T cells participate in an immunological network of interactions of idiotypes and anti-idiotypes is still unclear. Previous work has suggested that recognition of idiotype by T cells might require a processing step (6–8). We here present direct evidence that the idiotope of $\lambda 2^{315}$ has to be processed, since glutaraldehyde-fixed APCs present synthetic peptides but not native $\lambda 2^{315}$ to cloned idiotope-specific T cells. In this respect, T-cell recognition of immunoglobulin determinants appears not to be different from T-cell recognition of other proteins with regard to processing requirements (25). To show that a B cell processes the immunoglobulin that it produces itself, we have transfected the gene encoding the $\lambda 2^{315}$ idiotope-bearing light chain into B-lymphoma cell lines. These transfectants now express the $\lambda 2^{315}$ light chain together with the endogenous heavy chain on their cell surface. In addition the transfectants constitutively process and present this endogenous idiotope in the context of the class II

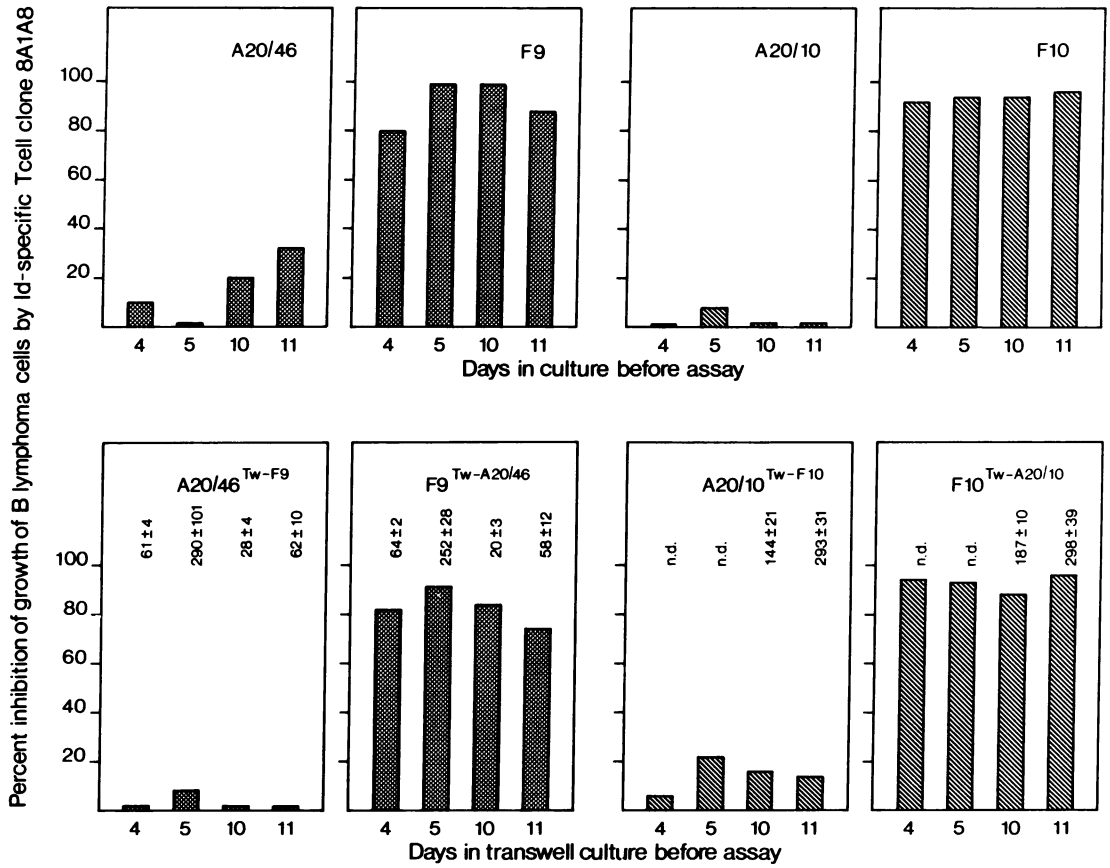

FIG. 3. Long-term cocultivation of transfectants and parental cells in diffusion chambers does not render the parental cells antigenic. Parental cells and transfectants were precultured alone (*Upper*) or cocultivated in diffusion chambers (*Lower*). During cocultivation, transfectant F9 or F10 cells were grown in the inside wells and the corresponding parental A20/46 or A20/10 cells were grown in the outside wells—e.g., A20/$46^{TW\text{-}F9}$ indicates A20/46 cells cultivated opposite (transwell) to F9 and $F9^{TW\text{-}A20/46}$ indicates F9 cells cultivated opposite to A20/46 cells in such diffusion chambers. Mean concentration of $\lambda 2^{315}$-containing molecules in supernatants from inside and outside wells at the time of cell harvest is displayed in ng/ml ± SD above the bars in *Lower*. Normal or cocultivated cells of these cultures were then used as APCs in the growth inhibition assay with the 8A1A8 T-cell clone. Radioactivity incorporated by B-lymphoma cells was in the range 12–510 × 10^3 cpm. SD of triplicates was generally below 20%. Free $\lambda 2^{315}$ added to A20/10 and irradiated 8A1A8 T cells at 10, 1, and 0.1 μg/ml induced 93%, 32%, and 6% inhibition, respectively.

molecule I-E^d to MHC-restricted idiotope-specific T-cell clones. This suggests that B-lymphoma cells and by extrapolation B cells display two forms of idiotype on their surface, one in the form of the native immunoglobulin and the other one in a processed form in the context of MHC molecules. While the former interacts with anti-idiotypic antibodies, as in the conventional network theory (1), the latter complex interacts with anti-idiotypic, MHC-restricted T cells in a different type of network interaction.

At least three different pathways could be involved in processing of the $\lambda 2^{315}$ light chain. First, $\lambda 2^{315}$ could be secreted and endocytosed. Second, surface immunoglobulin containing $\lambda 2^{315}$ could be internalized and processed. Third, a sample of intracellular $\lambda 2^{315}$ could be processed before ever reaching the cell surface. While the two latter possibilities are still open, the former possibility appears unlikely because the amount of $\lambda 2^{315}$ secreted into the supernatants by the transfectants is too small to induce presentation of idiotope when added exogenously to the parental B-lymphoma cells in long-term pulse experiments. This argument was confirmed by the diffusion chamber experiments (Fig. 3).

It has been suggested that only exogenous proteins are presented in the context of class II molecules (26–28). Our findings indicate that both exogenous and endogenous proteins can be processed and presented in the context of class II MHC antigens. However, we cannot exclude at the moment that the local concentration of $\lambda 2^{315}$ in the vicinity of a transfectant is so high that endocytosis of this $\lambda 2^{315}$ pool would be responsible for the antigenicity of the transfectant. This argument may be unambiguously settled by transfecting gene constructs in which the $\lambda 2^{315}$ chain cannot be secreted anymore. But even if local reuptake were responsible for the presentation of the $\lambda 2^{315}$ idiotope, the argument that a single B cell can process and present the immunoglobulin that it produces is still valid.

Our observations need to be extended to normal resting B cells *in vivo*—e.g., by use of mice transgenic for $\lambda 2^{315}$ gene. However, on the basis of the present findings, we suggest that normal B cells spontaneously process their immunoglobulin without interaction with ligand (antigen or anti-idiotypic antibodies). Such processed forms of immunoglobulin would associate with class II molecules and the complexes could be recognized on the surface of B cells by MHC-restricted T cells. However, as emphasized earlier (7, 8), there are several possible limitations: (*i*) some immunoglobulins might be resistant to processing (16); (*ii*) peptides resulting from processing might not associate with class II molecules; and (*iii*) T cells might be tolerant to peptides derived from germ-line-encoded immunoglobulin (29). If the latter point is correct, T cells may participate only in the regulation of somatically mutated B-cell clones. In addition, tolerance to peptides from germ-line-encoded variable (V) regions would influence the T-cell repertoire. However, due to a limited number of V-region gene segments (<1000), only a limited number of unique presentable immunoglobulin peptides are expected (maybe less than 10^4). Tolerance to such a number of peptides should not seriously delete the T-cell repertoire for foreign antigens.

Besides the spontaneous processing of endogenous immunoglobulin described above, other mechanisms might be important in T-cell-mediated, idiotype-related regulation of B cells. As suggested earlier (6, 9), ligation of surface immunoglobulin could induce or increase its processing. Furthermore, the ligand may be processed and presented itself as shown for antigen (30) and suggested for anti-idiotypic antibodies (7). Thus, an idiotype-bearing B cell could potentially communicate with antigen and anti-idiotypic antibodies via their reactive surface immunoglobulin and with different populations of MHC-restricted T cells via processed antigen, processed idiotype, or processed anti-idiotype in association with MHC molecules. Furthermore, the same B cell could interact with T cells which express T-cell receptors for which the native surface immunoglobulin of the B cell is specific (31). Finally, remember that other types of idiotype-specific T-cell clones have been described (32). It is obvious that regulation of a B cell by T cells *in vivo* is likely to be very complex.

Aside from network theory, our observations are interesting in a broader sense, since they indicate that cells process and present their self-proteins in general. This may be important for T-cell surveillance of altered self-antigens such as cancer antigens and autoantigens in autoimmune diseases.

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