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Immunology

Naive idiotype-specific CD4+ T cells and immunosurveillance of B-cell tumors

(tumor immunity/transgenic mice/plasmacytoma)

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ABSTRACT **The immunosurveillance hypothesis suggests that lymphocytes can recognize tumor-specific antigens expressed by transformed cells and initiate their elimination. Immunosurveillance implies that lymphocytes of naive phenotype can home to a tumor site and become activated by tumor-specific antigens. In this study, we have employed T-cell receptor transgenic mice as a source of naive, tumor-specific T cells. The transgenic, CD4+ T cells recognize a 91- to 101-residue fragment of the $\lambda 2^{315}$ immunoglobulin light chain presented by I-E^d class II molecules. Such naive, idiotype-specific, CD4+ T cells protected against tumor development of a class II negative plasmacytoma (MOPC315) and a class II positive B lymphoma (F9), which both secrete $\lambda 2^{315}$ immunoglobulin. Adoptive transfer experiments demonstrated that 2×10^6 lymph node cells were sufficient for protection against MOPC315. Depletion of T-cell subsets indicated that transgenic CD4+ cells were indispensable for tumor resistance. However, an additional role of CD8+ T cells is not ruled out. In contrast to the resistance against the secreting MOPC315 and F9 cells, transgenic mice were not protected against B lymphoma cells (F67), which do not secrete $\lambda 2^{315}$ but express a truncated $\lambda 2^{315}$ chain intracellularly. The results suggest that $\lambda 2^{315}$ is processed and presented by host antigen-presenting cells, which in turn activate naive, idiotype-specific T cells.**

Individual immunoglobulins carry unique antigenic determinants called idiotopes (Ids) in their variable (V) regions. These unique Id markers can function as tumor-specific antigens (TSAs) because immunization with immunoglobulin confers Id-specific resistance to a subsequent challenge with plasmacytomas and B lymphomas (1–3). However, the relative contribution of cellular and humoral immunity to Id-specific protection remains a matter of investigation (2–4).

Immunoglobulins can, like conventional extracellular antigens, be endocytosed and processed by antigen-presenting cells (APCs) (5, 6). In addition, by another pathway of processing, B lymphoma cells can process and present their own immunoglobulin (6, 7). In both cases, idiotypic peptides (Id-peptides) are presented on major histocompatibility complex (MHC) class II molecules to CD4+ T cells (5–7). Therefore, Id-peptides presented by class II molecules could function as TSAs when produced by malignant B cells. Indeed, tumor protection was found when cloned, Id-specific T-helper 1 (T_h1) or 2 (T_h2) cells were coinjected s.c. with either class II negative plasmacytoma cells (8) or class II positive B lymphoma cells (9). These studies (8, 9), however, left unanswered whether circulating CD4+ T cells of naive phenotype can home to a tumor region and respond to Id-peptides.

To approach this question, we have established BALB/c mice transgenic (TG) for an $\alpha\beta$ T-cell receptor (TcR) that recognizes an Id-peptide derived from the $\lambda 2^{315}$ light chain (L chain) of the MOPC315 myeloma protein (M315) (10). The Id-peptide comprises residues 91–101 of the $\lambda 2^{315}$ chain. Residues 94–96 are essential for recognition by the transgenic TcR (10); the $\lambda 2^{315}$ chain differs from the germ-line encoded $\lambda 2$ at these positions due to somatic mutations. The Id-peptide is presented on I-E^d class II molecules (5).

MATERIALS AND METHODS

Mice. TcR-TG mice (10) have been backcrossed to BALB/c for eight generations. Littermates were typed as TcR-TG or nontransgenic (N-TG) by Southern blot or PCR. The Fox Chase SCID mice (C.B-17/1cr scid/scid, severe combined immunodeficiency) were obtained from Bomholdt Gaard (Ry, Denmark). The SCID mice were kept in a barrier unit (specific pathogen free), while the TcR-TG mice were housed under conventional conditions (9).

Cells. The 4B2A1 T_h1 clone (5), the F9, F55, and F67 B lymphoma cell transfectants derived from A20/46 (6, 7), and the J558 plasmacytoma (American Type Culture Collection, ATCC) (11) have been described. MOPC315.4 (8) is a subclone of the MOPC315 plasmacytoma (ATCC) (12).

Antibodies and Flow Cytometry. The following monoclonal antibodies (mAbs) used were: RL172.4 (IgM) anti-CD4 (13); 3.155 (IgM) anti-CD8 (ATCC) (14); 20C9 (hamster IgG) anti-heat-stable antigen (HSA) (15); CTLA4Ig fusion protein (anti-B7) (16). Anti-CD4-phycoerythrin and anti-CD8-fluorescein isothiocyanate (FITC) were from Becton Dickinson. Flow cytometry was performed as described (17).

Measurement of M315. M315 in serum was measured by ELISA as described (8). The detection limit was 0.002 μg/ml.

Measurements of Cytokines. Lymph node (LN) cells from TcR-TG mice or 4B2A1 T cells (3×10^5 per ml) were stimulated essentially as described (18) by nonirradiated B tumor cells (3×10^5 per ml). Supernatants (SNs) were collected after 24 hr. Interleukins 2, 4, 5, 6, and 10 (IL-2, IL-4, IL-5, IL-6, and IL-10), interferon γ (IFN-γ), and tumor necrosis factor (TNF) were detected as described (18, 19). IL-3 was detected by a sandwich ELISA using anti-IL-3 (PharMingen, catalog no. 18011D) as capturing antibody, biotinylated anti-IL-3 (PharMingen, catalog no. 18022D) as

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Abbreviations: APC, antigen-presenting cell; ATCC, American Type Culture Collection; E:T, effector:target cell; HSA, heat-stable antigen; Id, idiotope; IFN-γ, interferon γ; IL, interleukin; LN, lymph node; mAb, monoclonal antibody; MHC, major histocompatibility complex; N-TG, nontransgenic; SCID, severe combined immunodeficiency; SN, supernatant; SP, spleen; TcR, T-cell receptor; TG, transgenic; T_h, T-helper; TNF, tumor necrosis factor; TSA, tumor-specific antigen; V, variable; L chain, light chain; FITC, fluorescein isothiocyanate.

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secondary antibody, and murine recombinant IL-3 (PharMingen) as standard.

***In Vitro* Proliferation Assay.** Mitomycin C-treated tumor cells (3×10^4 per well) were cultivated with LN cells or cloned T cells (2×10^5) for 64 hr with addition of 1 μCi of [*methyl*-^{3}H]thymidine (1 Ci = 37 GBq) for the last 16 hr (5).

***In Vitro* Growth-Inhibition Assay.** Inhibition of growth was detected by incubating tumor cells (10^4) with various numbers of irradiated (2000 rad; 1 rad = 0.1 Gy) LN cells or 4B2A1 T cells for 48 hr prior to a 16-hr pulse with 1 μCi of [*methyl*-^{3}H]thymidine as described (5). Where indicated, irradiated spleen (SP) cells (2.5×10^5) and $\lambda 2^{315}$ (20 μg/ml) were added.

Tumor Challenges. Anesthetized (9) littermates (6–28 weeks) from a BALB/c × Tcr-TG cross were injected s.c. in the interscapular region with F9 (1.25×10^6), F67 (3×10^5), F55 (1.25×10^6), MOPC315.4 (1.6×10^5), or J558 (8×10^4) cells. The numbers of injected tumor cells represent 2- to 4-fold of the numbers required to attain plateau-level tumor take in BALB/c mice (8, 9). Tumor development was monitored by weekly palpations; the week recorded refers to the first detection of a palpable tumor. Tumors invariably progressed.

***In Vitro* Depletion of T-Cell Subsets: Adoptive Cell Transfer.** LN cells were depleted of CD4$^+$ or CD8$^+$ T cells by complement-mediated cytotoxicity employing 3.155 or RL172.4 SN and guinea pig complement (GIBCO). Viable mononuclear cells were isolated over Lympholyte M (Cedarlane Laboratories). This treatment removed ≥98.5% of the relevant cell population according to flow cytometry analysis. Depleted or nondepleted LN cells were injected i.v. in the tail vein of SCID mice. Recipients were immediately challenged with 1.6×10^5 MOPC315.4 cells i.p.

Statistics. Data from *in vivo* tumor challenges were processed by life table calculations. The statistical analysis was performed by Petter Mowinckel (MedStat Research, Oslo) using a two-tailed log rank test (20).

RESULTS

Phenotype of TG LN Cells. In Id-specific TcR-TG mice, 15–20% of LN cells are CD4$^+$ T cells expressing the TG $\alpha\beta$ TcR (10). We first investigated the functional phenotype of such LN cells *in vitro*. As APCs, the following $\lambda 2^{315}$-producing tumor cell lines were employed. F9, derived from the BALB/c MHC class II positive A20/46 B cell lymphoma, is transfected with the $\lambda 2^{315}$ gene. F9 secretes small amounts of $\lambda 2^{315}$ (up to 0.5 μg/ml of SN) and strongly activates Id-specific, CD4$^+$ T-cell clones (6, 7, 9). The transfectant F67 is also derived from A20/46 and expresses a truncated $\lambda 2^{315}$ protein with a KDEL signal causing retention in the endoplasmic reticulum. The truncated $\lambda 2^{315}$ protein is only expressed intracellularly; nevertheless, F67 presents Id $\lambda 2^{315}$ to *cloned* T cells at an intermediate level (7, 9). The plasmacytoma MOPC315.4 secretes up to 12 μg of M315 myeloma protein (IgA, $\lambda 2^{315}$) per ml into SN. MOPC315.4 is MHC class II negative and does not stimulate Id-specific T cell clones unless professional APCs of the *H-2^d* haplotype are added (8). As negative controls, a mock transfectant of A20/46 (F55) and the J558 plasmacytoma (IgA, $\lambda 1$) were employed.

LN cells taken *ex vivo* from TcR-TG mice responded to F9 by proliferation (Fig. 1 *Top*), production of IL-2 (Table 1), and a weak inhibition of the growth of F9 at the highest E:T cell ratio in a 64-hr assay (Fig. 1 *Middle*). No short-term cytotoxicity was observed in a 5-hr ^{51}Cr-release assay (data not shown). TG LN cells also responded to F67, but weaker than to F9, and no growth inhibition was detected (Fig. 1, Table 1). No TG LN cell responses were observed to MOPC315.4, even in the presence of added SP APCs and additional $\lambda 2^{315}$ (Fig. 1 *Bottom* and Table 1, upper half).

In contrast to the TcR-TG LN cells, cloned 4B2A1 T$_h$1 cells, from which the TcR-TG is derived, are strongly growth inhibiting (Fig. 1), are cytotoxic in ^{51}Cr-release assays (9), and secrete both TNF and IFN-γ (18). Another difference, described earlier (8), is that TcR-TG LN cells express the MEL-14 homing receptor, typical for naive T cells (21), while 4B2A1 cells do not.

Taken together, the phenotype of the TcR-TG LN cells is in agreement with characteristics of naive class II restricted T cells in normal mice (21) and mice transgenic for other TcR (22–24). Our TcR-TG mice thus provide an appropriate system to study the role of naive T cells in immunosurveillance.

Tumor Challenges in TcR-TG Mice. TcR-TG and N-TG littermates were injected s.c. with the various tumor cell lines. TcR-TG mice were protected against the class II positive F9 B lymphoma ($P < 0.01$) and, importantly, against the class II negative MOPC315.4 plasmacytoma ($P < 0.01$) (Fig. 2). Both of these tumor cell lines secrete $\lambda 2^{315}$. The protection was transgene-specific, since N-TG mice developed F9 and MOPC315.4 tumors. The resistance was also Id-specific, because TcR-TG mice were not protected against F55 and J558 (Fig. 2).

In contrast to F9 and MOPC315.4, F67 tumor cells, which only express a truncated $\lambda 2^{315}$ protein intracellularly, were not eradicated in the TcR-TG mice (Fig. 2). This was surprising since F67 elicited a weak response in naive TcR-TG LN cells *in vitro* (Fig. 1, Table 1). Furthermore, the T$_h$1 clone 4B2A1 has previously been shown to protect against F67 in a Winn assay (9). One explanation for the lack of protection could be that F67 tumors in TcR-TG mice represented

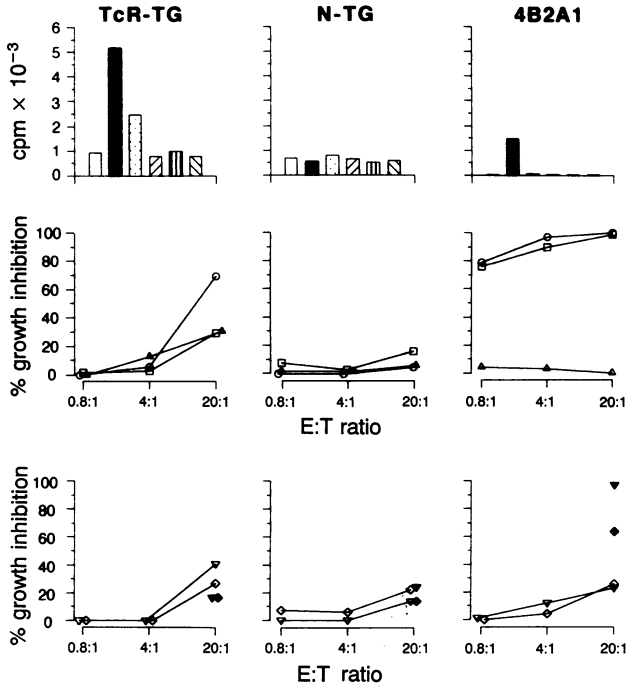

FIG. 1. Unprimed LN cells from TcR-TG mice proliferate but poorly inhibit the growth of tumor cells *in vitro*. The sources of responding cells (TcR-TG, N-TG, and 4B2A1 cloned T cells) are indicated above each column. (*Top*) Proliferation in response to mitomycin C-treated tumor cell lines. ■, F9; ▨, F67; ▨, F55; ▥, MOPC315.4; ▧, J558; □, medium. (*Middle*) Growth inhibition of various B lymphoma cell lines. ○, F9; □, F67; △, F55. (*Bottom*) Growth inhibition of plasmacytoma cell lines. ▽, MOPC315.4; ▼, MOPC315.4 plus BALB/c SP cells plus $\lambda 2^{315}$; ◇, J558; ◆, J558 plus BALB/c SP cells plus $\lambda 2^{315}$. E:T ratio, effector:target cell ratio.

Table 1. Cytokine secretion by lymphoid cells from TcR-TG mice and from reconstituted SCID mice surviving a MOPC315.4 challenge

Responder cells	APCs	IL-2, units/ml	IL-3, ng/ml	IL-4, units/ml	IFN-γ, ng/ml
TcR-TG*	F9	4.0	0	0	0
	F67	0.9	0	0	0
	F55	0	0	0	0
	MOPC315.4	0	0	0	0
	J558	0	0	0	0
SCID†	F9	38	0.8	0.90	26.4
	F67	35	1.0	0.24	1.6
	F55	0	0	0	0
	MOPC315.4	4	0	0.03	0
	J558	0	0	0	0

*LN cells were taken from nonmanipulated, adult TcR-TG mice.
†SCID mice reconstituted with TcR-TG LN cells (see experiment 2, Table 2), and surviving an i.p. injection of MOPC315.4, were donors of a mixture of LN and SP cells 25 weeks after tumor cell challenge. It was necessary to mix the cells (≈1:3) to obtain enough cells for the various assays. No IL-5, IL-6, TNF, or IL-10 was detected.

selected variants not presenting the Id-peptide. Arguing against this possibility, tumor cells taken *ex vivo* from TcR-TG mice injected with F67 still expressed I-A^d and I-E^d molecules and were recognized by cloned 4B2A1 T cells in growth-inhibition assays (data not shown).

Adoptive Transfer Experiments. To define the number of cells required for effective immunosurveillance, LN cells from TcR-TG or N-TG mice were injected i.v. into SCID mice. Reconstituted SCID mice were immediately injected with a tumorigenic dose of MOPC315.4 cells i.p. Only cells transferred from TcR-TG mice could protect against tumor development (Table 2, experiment 1). As few as 2×10^6 LN cells, corresponding to about $3–4 \times 10^5$ $CD4^+$ T cells expressing the transgenic αβ TcR, were sufficient for resistance (Table 2, experiment 2). Removal of $CD4^+$ T cells prior to transfer completely abrogated the MOPC315 resistance; however, depletion of $CD8^+$ cells also had a partial effect (Table 2, experiment 3).

While protected mice expressed no or minimal amounts of M315 myeloma protein, serum from tumor-bearing SCID mice reconstituted with subprotective numbers of TcR-TG cells contained large amounts of M315 (1.3–5.0 mg/ml)

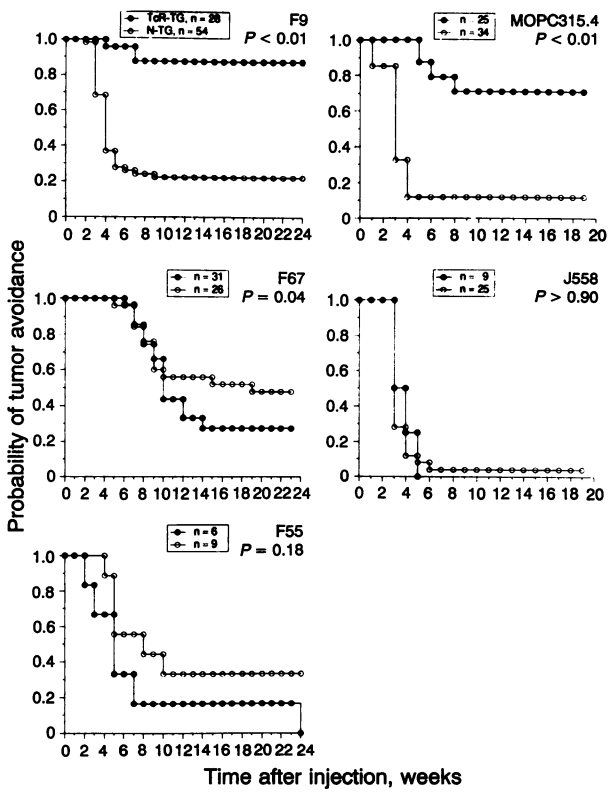

FIG. 2. TG mice are specifically protected against Id-secreting B-cell tumors. TcR-TG (●) and N-TG (○) littermates were injected s.c. with the indicated cell lines, and the occurrence of tumors was recorded. The diagrams represent Kaplan–Meier plots (20). Seven independent experiments were performed, three for F9 and one for each of the other tumor cell lines.

(Table 2). Furthermore, 28 of 28 tumor cell lines cloned from such mice still secreted M315 (data not shown).

SCID mice reconstituted with a sufficient number of TcR-TG LN cells, and therefore surviving a MOPC315.4 injection, expressed the TG TcR on $CD4^+$ (7–15%) and $CD8^+$ (3–11%) cells in their SP and LN (data not shown). LN and SP cells from surviving SCID mice were tested in functional assays 16–25 weeks after tumor challenge. Compared to

Table 2. Transfer of LN cells from TcR-TG mice conveys protection against MOPC315.4 tumors in SCID mice recipients

Exp.	Donor mice*	Transferred cells†	Tumor development‡	M315 in serum,§ μg/ml
1	TcR-TG	LN + SP	0/5	<0.002, <0.002, <0.002, <0.002, 32
	N-TG	LN + SP	6/6	1697, 1428, 400, 1314, 3885, 5369
2	TcR-TG	LN 2×10^4	2/2	3711, 5051
	TcR-TG	LN 2×10^5	2/2	3421, 1307
	TcR-TG	LN 2×10^6	0/2	<0.002, <0.002
	TcR-TG	LN 2×10^7	0/2	<0.002, <0.002
	N-TG	LN 2×10^7	2/2	3388, 7103
3	TcR-TG	$CD4^-$ LN 3×10^6	6/6	122, 1954, 2060, 677, 1355, 6378
	TcR-TG	$CD8^-$ LN 3×10^6	4/6	1591, 527, 149, 845, <0.002, <0.002
	N-TG	$CD8^-$ LN 3×10^6	3/3	809, 1081, 2717

*Adult (9–25 weeks old) TcR-TG and N-TG littermates were donors of inguinal, axillary, and cervical LN and SP cells.
†The number of donor cells injected i.v. into SCID recipients is indicated. In experiment 1, one LN and SP equivalent was injected per SCID mouse. In experiment 3, LN cells were depleted of $CD4^+$ or $CD8^+$ cells *in vitro* prior to transfer.
‡SCID recipients were injected i.p. with MOPC315.4 cells (1.6×10^5) immediately after receiving the donor cells i.v. The mice were examined twice weekly for 9–25 weeks for development of tumors and/or ascites.
§M315 in serum was measured at the time of killing of mice with progressive tumors or at the end of the experiment in animals free of disease.

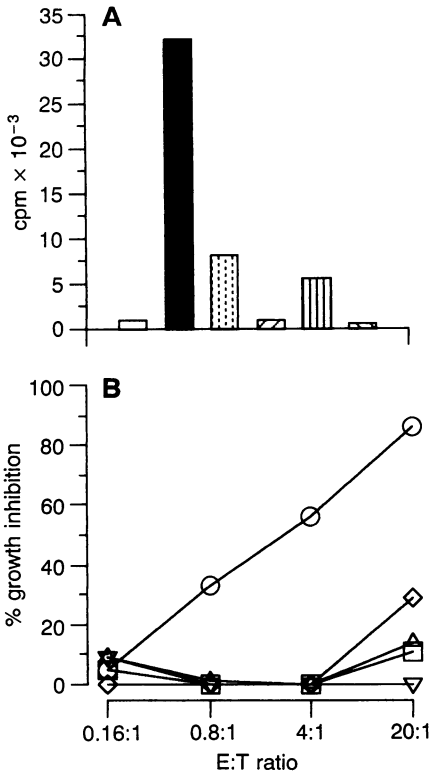

FIG. 3. *In vitro* effects of lymphoid cells from reconstituted SCID mice surviving a MOPC315.4 challenge. Surviving mice (see experiment 2, Table 2) were donors of a mixture (see text, Table 1) of LN and SP cells. (*A*) Proliferation of the SCID cells in response to mitomycin C-treated tumor cells. ■, F9; ▨, F67; ▨, F55; ▮, MOPC315.4; ▧, J558; □, medium. (*B*) Percent growth inhibition of the various tumor cell lines, effected by the SCID cells. ○, F9; △, F55; □, F67; ▽, MOPC315.4; ◇, J558.

naive TcR-TG LN cells (Fig. 1), increased growth inhibition and proliferation (Fig. 3) and lymphokine secretion (Table 1) were observed. The lymphokine profile had changed in that both IL-4 and IFN-γ were detected, and even MOPC315.4 cells induced a weak secretion (Table 1). The response to MOPC315.4 was presumably caused by APCs being sensitized by secreted M315 *in vitro* (8) and by more avid responses in secondary T cells compared to naive cells.

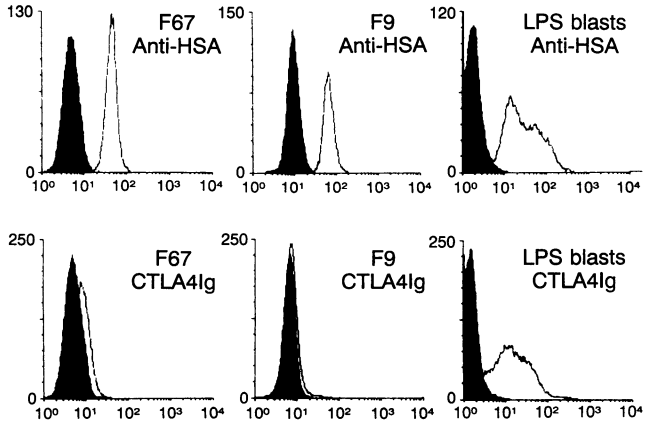

FIG. 4. Expression of HSA and CTLA-4 ligands (B7-1 and B7-2) on F67 and F9 cells. Cells were stained with the HSA-specific 20C9 hamster mAb or the CTLA4Ig fusion protein. Binding was detected by FITC-conjugated secondary reagents. Stainings with negative control immunoglobulin (hamster mAb H57 anti-TcR β or human IgG1 myeloma protein, respectively) are shown in black. LPS, lipopolysaccharide.

Expression of Costimulatory Molecules on Tumor Cells. Deficiency of costimulatory molecules required for activation of naive $CD4^+$ T cells could possibly explain the lack of protection against F67 cells in TcR-TG mice. Flow cytometry analysis (Fig. 4) revealed that both F67 and F9 cells expressed HSA, which has been described to be costimulatory (15). However, F67 only marginally expressed costimulatory CTLA-4 ligands (16) (B7-1 and B7-2) (Kolmogorov–Smirnov statistics, $P < 0.01$), while F9 (Fig. 4) and F55 (data not shown) hardly expressed any CTLA-4 ligands at all. Neither MOPC315.4 nor J558 was stained by CTLA4Ig. The former, however, expressed HSA (data not shown).

DISCUSSION

Since the original observation that immunization with the M315 myeloma protein induces an Id-specific protection to a subsequent MOPC315 challenge (1), the mechanisms for such Id-specific tumor immunity have been sought (reviewed in refs. 2–4). Anti-Id antibodies undoubtedly have an effect (2, 3), while the role of Id-specific T cells has been more elusive (4, 8, 9, 25).

Participation of Id-specific T cells was suggested by the finding that immunization with the V_L fragment of M315, emulsified in complete Freund's adjuvant, induces a partial protection against MOPC315 (26). However, immunization with $V_L{}^{315}$ elicits not only anti-Id T cells (26) but also anti-V_L antibodies (27). Furthermore, it may be argued that use of adjuvant, which efficiently induces effector cells, does not mimic physiological immunosurveillance of tumor cells.

To bypass these problems, we have established TcR-TG mice that possess high numbers of $CD4^+$ T cells recognizing an Id-peptide (residues 91–101) of the $\lambda 2^{315}$ chain, presented by I-E^d class II molecules (10). As shown, these TG T cells appeared to be of naive phenotype. It was therefore possible to study the role of naive, Id-specific T cells in immunosurveillance in the absence of preformed anti-Id antibodies. The results show that the mice were specifically protected against $\lambda 2^{315}$-secreting tumors.

TcR-TG mice are, however, unphysiological in the sense that they harbor an unnatural high number of specific T cells. We therefore performed adoptive transfer experiments that demonstrated that 2×10^6 LN cells were sufficient for protection. This corresponds to about 4×10^5 TG $CD4^+$ cells and 2×10^5 $CD8^+$ cells. As expected from the class II restriction of the TcR (10), $CD4^+$ T cells appeared to be absolutely required for tumor resistance. More surprisingly, elimination of $CD8^+$ cells prior to transfer partially reduced the resistance. This could be due to a need of $CD8^+$ cells for proper homing of $CD4^+$ cells in reconstitution of SCID mice (28). Alternatively, the $CD8^+$ T cells, due to expression of endogenous TcR α genes (10), could recognize some unknown peptide presented by class I molecules on the tumor cells. However, this appears unlikely because TcR-TG mice homozygous for the *scid* mutation are resistant to MOPC315 tumors; such TcR-TG *scid* mice have very few $CD8^+$ cells, all of which lack expression of endogenous TcR α chains (B.B., G.F.L., and Z.D., unpublished results).

The outcome of a tumor challenge appears to depend on the ratio of TG T cells to tumor cells. Too few transferred LN cells, or too many MOPC315 cells injected (unpublished results), resulted in plasmacytomas. These plasmacytomas invariably produced M315, even at the single cell level. We did not see MOPC315 variants producing only L chains, as commonly found in tumors obtained from M315-immunized mice (1). Thus, T cells may simply lose the battle against wild-type MOPC315, while anti-Id antibodies may select variants.

A challenge with MOPC315 obviously represents an antigenic stimulus for TG T-cell differentiation. Lymphoid cells

of reconstituted SCID mice, surviving a MOPC315 injection, had a more differentiated pattern of lymphokine secretion (IFN-γ, IL-4) (21), proliferated more vigorously, and showed increased growth inhibition of tumor cells compared to naive, TG cells.

A MOPC315 challenge could also stimulate Id-specific B cells to produce anti-Id^{315} antibodies participating in the tumor elimination. However, while mice hyperimmunized with M315 had as much as 10 μg of anti-Id^{315} antibodies per ml in their serum, only low amounts of such antibodies (50–200 ng/ml) were found in 9 of 16 TcR-TG mice surviving a MOPC315 injection given 86 days earlier. However, 2 of 7 noninjected control mice also had similar low levels of M315-binding antibodies in their serum, and there was no significant difference between the two groups (Mann–Whitney test, $P = 0.41$) (B.B., G.F.L., and Z.D., unpublished results). This finding, however, does not rule out anti-Id antibodies early in the response to a MOPC315 challenge.

Previous experiments with cloned T cells have suggested that M315, secreted by the class II negative MOPC315, is processed and presented by professional APCs (8). The present study indicates that APCs displaying Id-peptides can activate not only cloned but also naive T cells. Possibly, even subclinical MOPC315 tumors could secrete enough M315 to efficiently Id-sensitize local APCs. Circulating, naive, CD4$^+$ T cells encountering such APCs might differentiate and initiate tumor elimination. Alternatively, remote APCs become Id-sensitized by circulating M315 and activate T cells; such activated T cells could in turn produce lymphokines detrimental to distant tumor cells.

An important question is whether processing and presentation by host APCs are mandatory for stimulation of the naive T cells or whether tumor cells can activate the T cells directly without participation of host APCs. The protection of TcR-TG mice against F9 could support either possibility. F9 secretes small amounts of $\lambda 2^{315}$. These amounts have previously been shown to be insufficient for sensitizing bystander APCs in diffusion chamber experiments *in vitro* (6). However, it is possible that $\lambda 2^{315}$ secreted by F9 could be sufficient to sensitize APCs *in vivo*. Alternatively, the high concentration of Id-peptide/I-E^d complexes on the F9 lymphoma cell surface (7) could directly activate naive T cells.

The lack of protection in TcR-TG mice against F67, which does not secrete $\lambda 2^{315}$ protein, may also be interpreted in several ways. F67 cells only induced a marginal proliferation and lymphokine secretion *in vitro*. Therefore, the relative weak APC activity of F67 could be insufficient to induce the naive, CD4$^+$ T cells into tumor-protective effector cells *in vivo*. One explanation might be that F67 cells express too few Id-peptide/I-E^d complexes (7) to efficiently activate naive T cells. To evaluate this possibility, transfectants that hyperexpress the truncated $\lambda 2^{315}$-KDEL protein must be obtained. Another explanation is that F67 cells, similar to the parental A20 cells (29, 30), might be deficient in constitutive expression of costimulatory molecules required for activation of naive—but not cloned—CD4$^+$ T cells (31). Consistent with this idea, we have previously found that F67 induces cytotoxicity in cloned T cells *in vitro* (7, 9) as well as *in vivo* (9). Flow cytometry analysis revealed that F67 cells convincingly express HSA, while the expression of B7-1 and B7-2 antigens, recognized by CTLA4Ig (32, 33), is very weak but nevertheless significant. Perhaps this level of expression of costimulatory molecules is insufficient for activation of naive T cells. If so, secretion of $\lambda 2^{315}$ molecules and presentation by host APCs may actually be mandatory for differentiation of the naive T cells into tumor-protective effector cells in our system. Others have recently described that tumor cell expression of the B7 molecule greatly enhances tumor-protective CD8$^+$ (34, 35) and CD4$^+$ (36) T-cell responses.

In general terms, TSA secreted by tumor cells lacking costimulatory signals or class II molecules themselves might sensitize professional APCs, which are especially potent at activating naive CD4$^+$ T cells. CD4$^+$ T cells could have a decisive role in regulating anti-tumor activity in macrophages, CD8$^+$ T cells, and B cells (37). Nevertheless, immunosurveillance often fails. A major challenge will be to find out how the failure can be remedied.

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