T Cells from Late Tumor-bearing Mice Express Normal Levels of p56^{lck}, p59^{fyn}, ZAP-70, and CD3ζ Despite Suppressed Cytolytic Activity

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Summary

Loss of T cell–associated signal transduction molecules has recently been implicated in immune suppression in tumor-bearing hosts. In the present study, we have examined this and related phenomenon extensively in a large number of tumor-bearing mice, analyzed individually. Splenic T cells from tumor-bearing mice were isolated and characterized with respect to the following: (*a*) levels of three tyrosine kinases, p56^{lck}, p59^{fyn}, and ZAP-70; (*b*) expression of CD3-ζ; (*c*) alloreactive responses; and (*d*) antigen-specific responses. Contrary to recent reports, T cells from tumor-bearing mice were observed to express normal levels of *lck*, *fyn*, ZAP-70, and CD3-ζ. Further, T cells showed healthy alloreactive and antigen-specific responses until ~3 wk after post tumor challenge, when the tumors constituted ~20% of the body weight. Alterations with respect to some parameters were observed only in mice that had been bearing larger tumors for a considerably longer period. As human tumors are unlikely to grow to such large sizes (e.g., >20% of the total body weight), the significance of the alterations in T cell expression of *lck*, *fyn*, ZAP-70, or CD3-ζ in the immune status of cancer patients is unclear. Altogether, these results indicate that alterations in T cell signal transduction molecules do not account for the profound tumor-specific suppression observed during tumor growth.

The escape of immunogenic tumors from host immune response has long defined the central paradox of tumor immunology, and many mechanisms by which a primary tumor evades destruction have been suggested (1–4). Most recently, efforts to explain immunological suppression during tumor growth have focused on the apparent molecular defects in T cells in tumor-bearing hosts (5–9). These studies have suggested that down-regulation of the tyrosine kinases, p56^{lck}, p59^{fyn}, ZAP-70, and the ζ chain associated with both CD3 and CD16, could contribute to impaired function of T cells and NK cells isolated from tumor-bearing mice and patients.

To develop a broad understanding of the immunological alterations during progressive tumor growth, we have examined individual mice for the following: (*a*) levels of expression of p56^{lck}, p59^{fyn}, ZAP-70, and CD3-ζ; (*b*) the ability to generate an alloreactive response; and (*c*) the ability to generate an antigen-specific response in T cells from naive and tumor-bearing mice. We have done so in four different chemically and UV-induced tumors of two haplotypes. Our results indicate that the known alterations in T cell structure and function during progressive tumor growth are quite subtle except at the very end stages of disease, and they do not account for the profound tumor-specific suppression seen during tumor growth.

Materials and Methods

Animals and Tumor Cells. BALB/cJ and C57Bl/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME), and C3H-HeN mice were from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Meth A, CMS4, CMS5, UV-6139 (10), rat basophilic leukemia (RBL) (gift of Richard Klausner), CT-26, B/c-N, and B/c-NP were maintained in vitro in DMEM with supplements as described (11).

Tumor Challenges and Immunization. Mice were challenged and immunized according to Srivastava et al. (11).

Mixed Lymphocyte Reactions (MLRs),[1] *Mixed Lymphocyte Tumor Cultures (MLTC), and Cytotoxicity Assays.* MLRs, MLTCs, and ^{51}Cr release assays were carried out as described. (12).

Purification of T Cells and FACS® Analysis. T cells were positively selected from splenocytes using magnetic bead–coupled anti-Thy1.2 antibody and a cell sorting system (MACS, Miltenyi Biotec Inc., Sunnyvale, CA). Purity of enriched T cells (>95%) was assessed by FACScan® (Becton Dickinson & Co., Cockeysville, MD), using αCD3ε antibody (clone 145-2C11; Pharmingen, San Diego, CA).

Immunoblotting and Immunoprecipitation. Lysates of T cells or RBL tumor cells were blotted and immunoprecipitated as described (11). Blots were probed with antiserum to *lck* and *fyn*,

[1] *Abbreviations used in this paper:* MLR, mixed lymphocyte reaction; MLTC, mixed lymphocyte tumor culture; RBL, rat basophilic leukemia.

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polyclonal antibody to ZAP-70 (Upstate Biotechnology, Inc., Lake Placid, NY), or antiserum to CD3-ζ (a kind gift of Dr. Richard Robb, Oncotherapeutics, Inc., Cranbury, NJ) and developed with peroxidase-conjugated antibody to rabbit by enhanced chemiluminescence (Amersham Corp., Arlington Heights, IL).

Results

A number of independent immunological parameters relating to T cell structure and function were monitored during progressive tumor growth. Splenic T cells from tumor-bearing mice were isolated and analyzed for the following: (*a*) levels of expression of three tyrosine kinases, *lck*, *fyn*, and ZAP-70; (*b*) expression of CD3-associated ζ chain; (*c*) alloreactive responses; and (*d*) antigen-specific T cells responses.

Expression of Signal Transduction Molecules, lck, fyn, ZAP-70, and CD3-associated ζ in T Cells during Tumor Growth. BALB/cJ (H-2^d) mice were challenged intradermally with antigenically distinct methylcholanthrene-induced fibrosarcomas, Meth A, CMS4, or CMS5. All tumors grow progressively and kill their hosts between 6 and 12 wk after tumor challenge, depending on the tumor. Mice were killed periodically, and splenic T cells were enriched by positive selection using magnetically coupled αThy1.2 antibody and the MACS cell sorting system. T cells were consistently >95% pure, as analyzed by FACScan® using αCD3ε antibody. Splenic T cells from naive mice were similarly isolated and used as controls in each experiment. T cell lysates applied to SDS-PAGE, transferred to polyvinyldifluoride membrane, and probed with antisera to each of four signal transduction molecules, *lck*, *fyn*, Zap-70, and CD3-ζ. In total, ~100 mice were examined individually.

lck is observed to be present in splenic T cells of naive mice and in nearly all tumor-bearing mice. Only at the most extreme stages of tumor growth (day 45) is *lck* observed to be absent in CMS5-bearing mice (Fig. 1). This loss is similarly seen in CMS4 and Meth A bearers at this stage (data not shown). T cells were similarly isolated by positive selection from two C3H-HeN (H-2^k) mice bearing large UV-6139 tumors (day 55). *lck* is substantially decreased in the first mouse and is missing in the second, as compared with a naive control (Fig. 1). These studies have been carried out in >50 mice individually. They indicate that *lck* expression in T cells in tumor bearing varies moderately from mouse to mouse, and that significant loss occurs only during the very late stages of the tumor-bearing period.

fyn is observed to be present in BALB/cJ mice bearing 10- and 45-d tumors at levels comparable to control non–tumor-bearing mice (Fig. 2). In additional experiments with these tumors, *fyn* was analyzed at 8, 16, 26, 30, 35 and 45 d after tumor challenge, and it is observed to be present in all T cells isolated (data not shown). In contrast to these results with BALB/cJ mice, *fyn* was observed to be absent in T cells isolated from two late-stage (day 55) UV 6139–bearing C3H-HeN mice (Fig. 2).

The recent discovery of a novel tyrosine kinase, ZAP-70, has added another level of complexity to TCR signaling (13, 14). While a defect in ZAP-70 has been implicated in human severe combined immunodeficiency (15) and in selective T cell deficiency (16), no previous reports have documented the effects of tumor burden on this tyrosine kinase. Splenic T cells were isolated by positive selection from Meth A and UV 6139 tumor–bearing mice. In Meth A bearers, T lymphocytes from mice bearing 10-d-old tumors show ZAP-70 levels comparable with those of control mice. Mice bearing 34- or 45-d-old tumors, however,

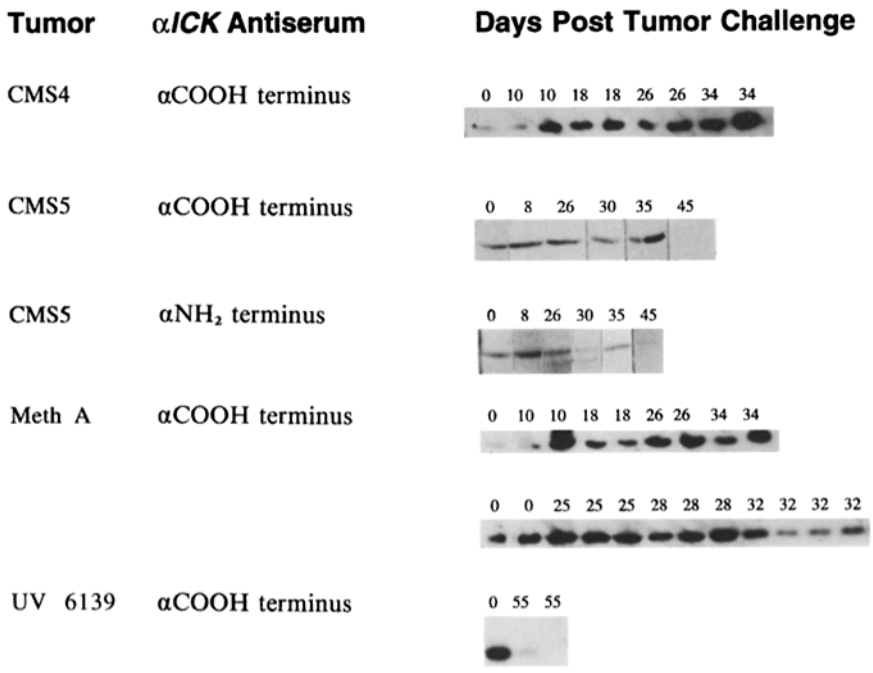

Figure 1. Expression of p56^{lck} in splenic T cells isolated from tumor-bearing mice. T cells were isolated by positive selection from CMS4, CMS5, Meth A, and UV 6139 tumor bearers (see Materials and Methods). T cell purity was >95%. Cell extracts were subjected to SDS-PAGE were immunoblotted, and were probed with antiserum to the COOH or NH_2 terminus of *lck*. Day 0 represents T cells isolated from naive mouse splenocytes.

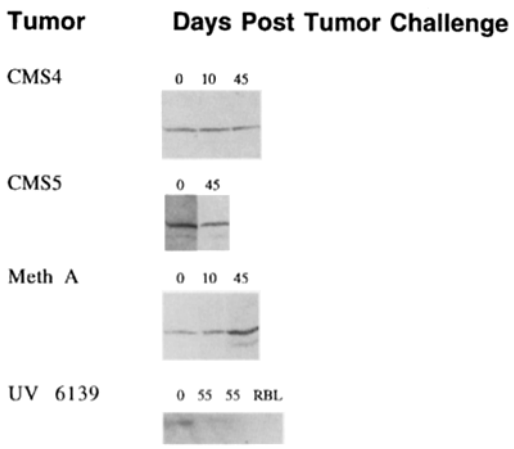

Figure 2. Expression of $p59^{fyn}$ in splenic T cells isolated from tumor-bearing mice. For experimental details, see the legend to Fig. 1 and the Materials and Methods section. All immunoblots were probed with antiserum to *fyn* synthetic peptide. RBL is a negative control for *fyn*.

show a significant decrease in ZAP-70 expression. In mice bearing UV 6139 (day 55), ZAP-70 is observed to be completely absent (Fig. 3).

CD3-ζ has been the subject of extensive reports concerning T cell development in the thymus and T cell function in the periphery (17–21), thus generating particular interest regarding its role in T cell abnormalities in tumor bearers. Similar to analysis of *lck*, *fyn*, and ZAP-70, expression of CD3-ζ was examined at all stages of tumor growth, with emphasis on late tumor bearers. For initial experiments, the total T cell lysates were subjected to 14% SDS-PAGE, immunoblotted, and probed with anti-ζ antiserum (Fig. 4 *A*). ζ was observed to be present in all lysates examined, including late CMS4 and Meth A tumor bearers (day 34). In two exceptional cases (2 out of 10), mice bearing early CMS4 and Meth A tumors (day 10) showed a decrease in ζ expression. Additional experiments focused on later stages of Meth A tumor growth, and ζ was observed to be present even in mice bearing 45-d tumors (data not shown).

These experiments did not address the possibility that ζ, although present, might dissociate from the CD3 complex,

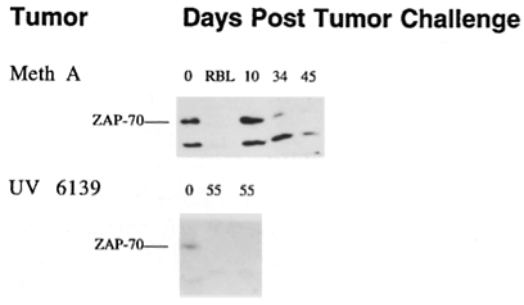

Figure 3. Expression of ZAP-70 tyrosine kinase in splenic T cells isolated from tumor-bearing mice. T cell lysates were prepared as described (Fig. 1 and Materials and Methods) and immunoblots probed with polyclonal antibody to ZAP-70. RBL is a negative control for ZAP-70.

thus disrupting the cascade of signaling events. To address this possibility in Meth A and CMS5 tumor bearers, the CD3 complex was precipitated with αCD3ε (clone 145-2C11), subjected to 14% SDS-PAGE, immunoblotted, and probed with anti-ζ antiserum (Fig. 4 *B*). Again, ζ was detected in all nonprecipitated lysates at all stages of tumor growth. In precipitated lysates, ζ is similarly detectable in Meth A tumor bearers through day 29, and in CMS5 tumor bearers through day 53. In very late–stage Meth A bearers (day 34), ζ is not detectable after precipitation, suggesting dissociation from the CD3 complex. Similar to the analysis of *lck*, expression of CD3-ζ was extensively analyzed in highly enriched T cells isolated from >50 mice. Altogether, these results demonstrate that, in nearly every mouse examined, ζ is not only present but remains associated with the CD3 complex. The cases where ζ is present but has dissociated from other CD3 components are observed only in T cells isolated from some mice with greatly advanced disease.

Figs. 1–4 show representative examples of expression of the various signal transduction–related molecules in T cells from tumor-bearing mice. However, these examples represent only a proportion of the mice examined. Table 1 shows the compiled results of analyses of expression of *lck*, *fyn*, and CD3-ζ in T cells from mice bearing Meth A, CMS4, CMS5, or UV-6139 at various stages of tumor growth. The results from all mice whose T cells were examined for expression of at least two of the three molecules are included in this table. It is obvious from this comprehensive and extensive compilation that, in general, T cells from mice bearing large tumors are competent with respect

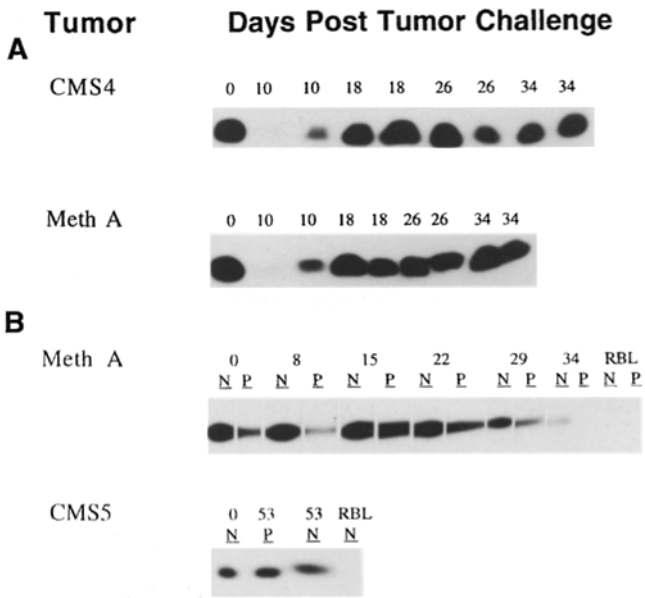

Figure 4. Expression of CD3-ζ in splenic T cells isolated from BALB/cJ tumor–bearing mice. Whole cellular extracts (*A*) or extracts first precipitated with monoclonal αCD3ε (*B*) were applied to SDS-PAGE, immunoblotted, and probed with α/ζ antiserum. *N* indicates nonprecipitated lysates, while *P* indicates precipitated lysates. RBL is a negative control for ζ.

Table 1. *Comparison of Expression of Signal Transduction Molecules in T Cells from Mice Bearing Meth A, CMS4, CMS5, or UV6139 Tumors*

Tumor	Days of tumor burden	Average tumor diameter*	Presence of:** *lck*	*fyn*	CD3-ζ	Tumor	Days of tumor burden	Average tumor diameter	Presence of: *lck*	*fyn*	CD3-ζ
		mm						*mm*			
Meth A	0	0	+	++	+++	CMS4	0	0	+	++	+++
	0		++	+	+++		8		++	++	nd
	0		++	++	nd		10	6.1	+	+	−
	0		++	++	nd		10		++	+	+
	8		++	++	nd		10		++	++	nd
	10	6.0	+	+	−		16		+	++	nd
	10		++	+	+		18	8.0	++	+	+++
	10		++	++	nd		18		++	+	+++
	18	15.5	++	++	+++		26	11.0	++	++	+++
	18		++	++	+++		26		++	++	++
	25	26.0	+++	+	+++		26		+	++	nd
	25		++	++	+++		30		+	++	nd
	25		++	+	++		34		++	++	+++
	26		++	++	+++		34		+++	++	+++
	26		++	++	++		35		−	++	nd
	26		++	++	nd		45		−	++	nd
	28		++	+	+++						
	28		++	++	+++	CMS5	0	0	+	++	+++
	28		+++	+++	+++		8	5.0	++	++	nd
	30		++	++	nd		26	14.0	++	++	nd
	32	27.3	++	++	+++		30		++	++	nd
	32		+	++	+++		35		++	++	nd
	32		+	++	+++		45		−	++	nd
	32		+	++	+++						
	34		++	++	+++	UV6139	0	0	++	+	nd
	34		+++	++	+++		55	25.0	+	−	nd
	35		−	++	nd		55		−	−	nd
	45		−	++	nd						

*Shown are representative tumor diameters.

**++ represents average expression, as detected by immunoblotting with specific antibody, as shown in Figs. 1–4, +++, two or more times higher than average expression; +, 50% or lower than average expression, −, no expression, nd, not determined.

to expression of the signal transduction–related molecules. Among mice bearing tumors for 25 d or longer, relative or absolute loss of *lck* was observed only in 2 out of 18 mice bearing Meth A, two out of eight mice bearing CMS4, one out of four mice bearing CMS5, and one out of two mice bearing UV6139. Relative or absolute loss of *fyn* was never observed in mice bearing Meth A, CMS4, or CMS5, but was detected in two mice bearing 55-d-old UV6139 tumors. Relative or absolute loss of CD3-ζ was never observed in mice bearing large tumors. These results demonstrate that the overwhelming majority of mice contain a normal complement of signal transduction molecules and that losses are very infrequent, even at advanced stages of tumor growth.

Loss of Alloreactivity During Tumor Growth. In addition to monitoring structural parameters (see Fig. 1–4), two functional properties of T cells from tumor-bearing mice were tested as a function of tumor growth, including alloreactive and antigen-specific responses. Three separate groups of BALB/cJ mice were challenged intradermally with CMS4, CMS5, or Meth A tumor cells. To assess alloreactivity, MLRs were established. Beginning 9 d after tumor challenge and at 10–14-d intervals thereafter, splenocytes from tumor bearers were divided equally and cul-

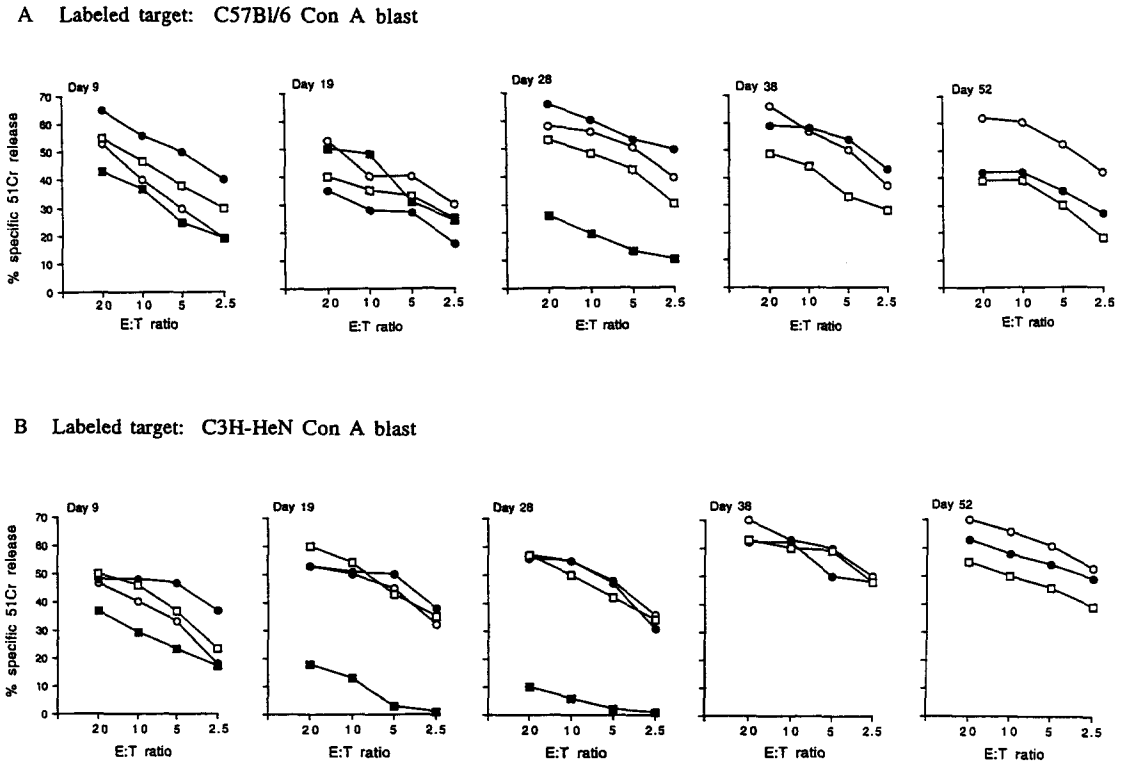

Figure 5. Loss of alloreactivity during tumor growth. Splenocytes from BALB/cJ tumor-bearing mice were prepared for MLRs as described. Mitomycin C–treated splenocytes from naive C57Bl/6 and C3H/HeN mice served as stimulator cells. CTL assays were performed on day 5 with ^{51}Cr-labeled target cells consisting of Con A–stimulated naive C57Bl/6 (*A*) or C3H-HeN (*B*) spleen cells. The number of days after tumor challenge when MLRs were established is indicated at the top left of each figure. Meth A tumor–bearing mice were not analyzed on day 38 or 52. (–○–), naive; (–●–), CMS4; (–□–), CMS5; and (–■–), Meth A.

tured with either mitomycin C–inactivated C57Bl/6 or C3H-HeN stimulator cells. On day 5, CTL assays were performed as described in Materials and Methods, with target cells consisting of Con A–stimulated C57Bl/6 (Fig. 5 *A*) or C3H-HeN (Fig. 5 *B*) splenocytes.

Splenocytes from mice bearing tumors up to ~3 wk showed normal levels of alloreactivity to C57Bl/6 and C3H-HeN stimulators in ^{51}Cr release assays (Fig. 5, *A* and *B*). In contrast, MLRs that included responding splenocytes from large Meth A tumor bearers (>3 wk) showed a 50% or greater loss of alloreactivity. This loss begins 19 d after tumor challenge in response to C3H-HeN stimulators, and on day 28 in response to C57Bl/6 stimulators. CMS4 and CMS5 tumor–bearing mice show a less dramatic loss of alloreactivity in these studies. By day 52, the response of these splenocytes to C57Bl/6 stimulators is reduced by 30% (Fig. 5 *A*), with only a slightly reduced response to C3H-HeN stimulators (Fig. 5 *B*). The modest loss of alloreactivity in CMS4 and CMS5 tumor bearers compared with Meth A tumor bearers correlates with slower growth kinetics of CMS4 and CMS5 tumors. In other experiments involving mice bearing larger CMS4 and CMS5 tumors, however, a loss of alloreactivity comparable to Meth A bearers is observed (data not shown).

To evaluate the antigen-presenting function of splenocytes isolated from tumor bearers, the responder and stimulator cells in MLRs were reversed. Splenocytes from mice with either small (day 8) or very large tumors (day 30) were treated with mitomycin C and used as stimulators, while splenocytes from naive mice served as responders. CTL assays revealed normal levels of cytotoxicity, suggesting that antigen-presenting function in splenocytes of tumor bearers is intact (not shown).

Loss of Antigen-specific T Cell Responsiveness During Tumor Growth. Loss of alloreactivity is observed only in late tumor–bearing mice (see Fig. 5), suggesting that, in mice bearing smaller tumors, T cell–mediated immunity is specifically suppressed with respect to the tumor implant, but not with respect to other antigens. This was tested by immunizing Meth A–bearing mice with the highly immunogenic BALB/c colon carcinoma line, CT-26, and assessing the ability of mice to generate a tumor-specific CTL response to CT-26 tumor. Two groups of mice were challenged intradermally with 1×10^5 Meth A cells staggered at a 14-d interval (Fig. 6, *bottom*). Mice received their first immunization with irradiated CT-26 cells either 6 d or 20 d after tumor challenge, and their second immunization 13 or 27 d after tumor challenge. Non–tumor-bearing mice were similarly immunized. 3 d after the last immunization, mice were killed and splenocytes were cultured with irradiated CT-26 stimulators at a ratio of 200:1. On day 6, CTLs were tested in a chromium release assay against CT-26,

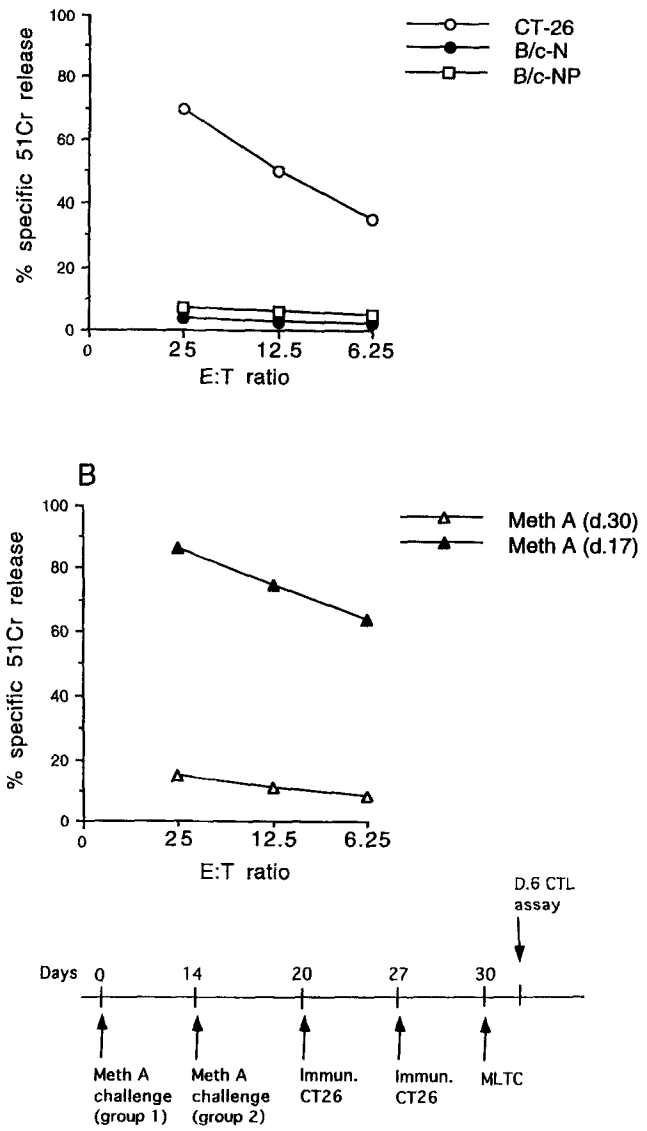

Figure 6. Suppression of tumor-specific reactivity to CT-26 tumor cells in mice bearing large Meth A tumors. Naive mice were immunized twice at weekly intervals with whole irradiated CT-26 cells, and MLTCs were established as described. On day 6, CTLs were tested against ^{51}Cr-labeled CT-26, B/c-N, and B/c-NP targets, as indicated (*A*). Meth A tumor-bearing mice were similarly immunized with CT-26 cells. The number of days after tumor challenge when MLTCs were established is indicated (*B*). The ^{51}Cr-labeled target cell is CT-26. The time course of tumor challenges and immunizations is indicated for each group of mice at the bottom of the figure.

B/c-NP (BALB/c fibroblast transfected with the NP gene of PR8 influenza virus), and B/c-N (parental, nontransfected fibroblast line) target cells. CTLs derived from non–tumor-bearing mice that were immunized with CT-26 show vigorous cytolytic activity against CT-26 target cells, but not against B/c-N or B/c-NP targets (Fig. 6 *A*). Splenocytes from mice bearing 17-d tumors at the time of MLTC also generate a vigorous CTL response to CT-26 (Fig. 6 *B*). This response is even greater than CTLs from the control non–tumor-bearing mice. In contrast, splenocytes from mice bearing 30-d tumors at the time of MLTC generate a negligible T cell response to CT-26. This suggests that T cells in mice bearing tumors up to ~3 wk are capable not only of responding broadly to alloantigens but are also capable of responding specifically to a syngeneic tumor antigen. However, both of these responses are soon lost in mice bearing tumors longer than 3 wk.

Discussion

There has been considerable interest recently in the observed loss of signal transduction molecules from T cells of tumor-bearing hosts, as reported in two murine tumor systems (5, 8, 9), and subsequently in human cancer patients (6, 7). Our results (Figs. 1–4 and Table 1) are apparently dissonant with these findings. First, it was observed that splenic T lymphocytes derived from the majority of BALB/cJ mice bearing CMS4, CMS5, or Meth A tumors, and from C3H-HeN mice bearing UV 6139 tumors, contained normal levels of the tyrosine kinases *lck* and ZAP-70, although some mice with greatly advanced tumors show a decrease or complete loss of expression. Second, it was observed that the tyrosine kinase *fyn* is present at normal levels in T cells isolated from all BALB/cJ tumor–bearing mice and is missing from T cells isolated from C3H/HeN tumor–bearing mice. Third, normal levels of CD3-ζ were detected in T cells isolated from all BALB/cJ tumor–bearing mice, although dissociation of ζ from the CD3 complex was observed at very late stages of tumor growth. We have examined T cells for their content of signal transduction molecules in four antigenically distinct tumor systems, comprising two haplotypes. These studies were done at various time points and involved >100 mice, individually examined. In total, these results suggest that the alterations in signaling molecules may not be prevalent with equal severity in all tumor systems and that a wide variety of mechanisms could contribute to the down-regulation of T cell–mediated immunity in tumor bearers.

We have considered the variation in experimental design that may have led to the significant differences between our results (presented here) and those in two earlier studies (5, 8). Experiments in the earlier reports appear to have involved pooling T cells from many mice, and they do not address variation in expression from host to host, as detected by us (see Figs. 1 and 4). Further, the earlier studies presented results only from naive and late tumor bearers, with only one mention of analysis of T cells at other time points (8). In contrast, our study involved inoculating large groups of mice with tumor cells, followed by analysis of signaling molecules on an individual mouse basis at many stages of tumor growth. It is possible that some of the observed defects in such molecules in tumor bearers (5, 8) could be attributed to normal fluctuation from host to host. For example, in our experiments in which T cells were isolated from individual mice bearing CMS4 or Meth A tumors for the same duration (i.e., days 10, 18, 25, 26, 28, 32, and 34), considerable variation in expression of *lck* was

in fact observed (Fig. 1). Interestingly, a recent report (9) showed no loss of CD3-ζ among the T lymphocytes derived from lymph nodes and intraepithelial lymphocytes of a late tumor–bearing mouse, although such loss was observed in splenic T lymphocytes. This observation underscores the fact that any alterations in TCR structure may not be systemic but may result from a specific host microenvironment created by some tumors but not others.

Numerous reports have demonstrated that loss of certain signal transduction proteins has deleterious effects on T cell function (16, 17, 22, 23). CD3-ζ chain was reported to be replaced by the Fcεγ chain in a recent study of splenic T cells isolated from C57Bl/6 mice bearing the MCA-38 colon carcinoma (5). The functional consequence of this substitution is not entirely clear, as normal lytic function of such cells in response to antigen stimulation has been demonstrated (24).

Normal alloreactive T cell responses were observed in BALB/cJ mice bearing tumors up to ~3 wk, although mice bearing tumors for longer periods demonstrate a decrease in alloreactivity. This loss has been observed in late tumor–bearing mice in two other systems (25, 26). It was also observed that mice bearing tumors at relatively advanced stages of growth (day 17) are capable of eliciting antigen-specific T cell responses to an unrelated tumor, and that only in mice bearing tumors 3–4 wk is this ability lost (Fig. 6). This phenomenon represents a paradox of immune suppression, in that the ability to generate a specific T cell response to another distinct tumor antigen is concurrent with the progressive growth of a highly immunogenic tumor in syngeneic hosts. This suggests that mice are specifically suppressed only with regard to the implanted tumor, and not with regard to other antigens. At very late stages of tumor growth, however, a shift from antigen-specific to generalized suppression occurs, such that mice are more severely immunocompromised.

Although this study examined a number of parameters associated with T cell structure and function in tumor-bearing hosts, it has not addressed the mechanisms by which tumor-specific suppression is manifested. Studies of R. J. North and I. Bursuker have shown that, as early as 9 d after tumor challenge, a suppressor T cell population proliferates, reaching maximum activity by day 16 (1, 2). Presumably, these suppressor T cells are fully functional with respect to the signaling molecules addressed in our study, in addition to retaining the ability to respond to alloantigens and other specific tumor antigens. The generation of this suppressor T cell population at very early stages of tumor growth is significant in that it suggests that alterations in the host immune response do in fact occur very soon after tumor transplantation; however, these alterations are most likely distinct from those alterations at later stages of tumor growth, observed in this study.

To the best of our knowledge, this is the first report that examines the immune response to tumor progression in individual mice, using multiple parameters, studied simultaneously in more than one tumor. In light of the observed maintenance of immunocompetence for the majority of the life span of tumor-bearing mice, it may prove fruitful to further study antigen-specific suppression at early stages of tumor growth, where the chances of permanently eradicating tumors greatly increase.

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Note added in proof: In a forthcoming study of four murine tumor systems (Franco, J., P. Gosh, R. Wiltrout, C. Carter, A. Zea, N. Momozaki, A. Ochoa, D. Longo, T. Sayers, and K. Komschlies. 1995. *Cancer Res.* In press), the authors show that granulocytes contaminating T cell preparations induce loss of T cell signaling molecules $p56^{lck}$ and CD3ζ, Although the authors do not provide data, they claim that this artifact does not account for loss of CD3ζ in human cancer patients. Another study (Wang, Q., J. Stanley, S. Kudoh, J. Myles, V. Kolenko, T. Yi, R. Tubbs, R. Bukowski, and J. Finke. 1995. *J. Immunol.* 155:1382–1392) shows a lack of loss of $p56^{lck}$, $p59^{fyn}$, ZAP-70, and CD3ζ in tumor-infiltrating lymphocytes of 29 patients with non-Hodgkin's B cell lymphomas.

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