Tumors Escape CD4⁺ T-cell-Mediated Immunosurveillance by Impairing the Ability of Infiltrating Macrophages to Indirectly Present Tumor Antigens

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Abstract

Tumors cells can escape cytotoxic CD8⁺ T cells by preventing MHC I display of tumor antigens. It is unknown how tumors evade CD4⁺ T-cell responses, but because many tumor cells lack MHC II expression, novel mechanisms would be required. We have investigated this issue in a model in which MHC II⁻/⁻ myeloma cells secrete a monoclonal Ig containing a V region L chain (Vₜ) epitope recognized by CD4⁺ T cells. Infiltrating macrophages process and present the secreted tumor antigen to Th1 cells, resulting in induction of macrophage cytotoxicity and apparent rejection of the tumor. Despite long-term tumor protection in Vₜ-specific T-cell receptor transgenic mice, we here describe that some myeloma cells persisted in a dormant state and, eventually, formed expanding tumors. Escape tumor cells maintained their secretion of complete (H⁺L) monoclonal Ig with unchanged sequence, while secretion of surplus free L chain was severely diminished. Although free L chains were efficiently processed and presented by tumor-infiltrating macrophages to CD4⁺ T cells, complete (H⁺L) monoclonal Ig was not. Forced overexpression of free L chain secretion reinstated tumor rejection. These results show that tumors can evade CD4⁺ T-cell-mediated rejection by impairing indirect presentation of tumor antigen by infiltrating macrophages. This occurs through a novel mechanism of immunoediting, in which modulation of the quaternary structure of the secreted tumor-specific antigen reduces its immunogenicity. Cancer Res; 75(16); 3268–78. ©2015 AACR.

Introduction

Previous strategies to induce effective antitumor immune responses have focused largely on cytotoxic CD8⁺ T cells that recognize tumor-specific peptides on MHC class I on the tumor cell surface. The efficacy of CD8⁺-mediated antitumor immune responses is limited by the commonly observed phenomenon of tumor escape, in which subsets of tumor cells become resistant to killing by effector CD8⁺ cells. Several mechanisms of tumor evasion have been described, including loss of tumor antigen synthesis or impaired loading on MHC class I molecules (1–3). This type of adaptation of residual tumor cells to an ongoing immune attack is commonly referred to as immunoediting (4).

Recent work has shown that tumor-reactive CD4⁺ T cells may significantly improve the efficacy of CD8⁺ T cells in adoptive T-cell therapy (5, 6). Moreover, CD4⁺ T-cell transfer may confer tumor eradication in the absence of CD8⁺ T cells (7–11). These results have lead to a growing interest in the use of CD4⁺ T cells in immunotherapy in humans (11, 12).

Antigen recognition by CD4⁺ T cells is dependent on antigen display on MHC class II, which is normally limited to professional antigen-presenting cells (APC). Although some tumor cells express MHC class II (8, 9) and have been shown to be killed by direct interaction with cytotoxic CD4⁺ T cells by granzyme B/fas ligand-mediated (13) mechanisms, a large number of tumor cells lack detectable surface-bound MHC class II in vivo. Engagement of CD4⁺ T cells in such cases is therefore dependent on antigen uptake and display on APCs. Several groups have demonstrated that antitumor CD4⁺ T-cell responses can protect against MHC II negative (MHC II⁻/⁻) tumor cell types (14, 15), supporting the notion that indirect display of tumor-specific antigens on APCs can mediate tumor rejection. Several effector mechanisms have been implicated in such processes, including activation of macrophages (15, 16) or NK cells (14), or the induction of antiangiogenic cytokines such as IFNγ (10).

We have previously demonstrated that CD4⁺-mediated rejection of MHC II⁻/⁻ myeloma cells is dependent on antigen secretion by the tumor cells, and is mediated by uptake and presentation of secreted tumor antigens on APCs within the tumor stroma as well as in draining lymph nodes (16–18). The killing of tumor cells occurs indirectly through Th1-mediated activation of tumor-infiltrating macrophages into M1-like cells with cytotoxic properties (16, 19). This indirect Th1/M1 mechanism of tumor cell killing is critically dependent on secretion.

References

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of the tumor antigen (18). Because such indirect recognition by CD4+ T cells uncouples antigen presentation from the tumor cell itself, evasion of the immune response would be expected to involve either impaired secretion, mutations in the secreted tumor antigen, or impaired antigen uptake/presentation by host APCs. We here report evidence for the occurrence of the latter.

Multiple myeloma cells secrete monoclonal Ig (myeloma protein) that often contains unique amino acid sequences due to VDJ recombination and somatic hypermutation. Such nongermline sequences constitute attractive targets for immunotherapeutic protein) that often contains unique amino acid sequences due to VDJ recombination and somatic hypermutation. Such nongermline sequences constitute attractive targets for immunotherapeutic interventions in B-cell malignancies. We have previously described the generation of a T-cell receptor-transgenic (TCR-Tg) mouse that harbors CD4+ T cells specific for a mutated tumor-specific epitope (idiotope, Id) in the V_L region of the M315 myeloma protein produced by the MOPC315 myeloma cell line (20, 21). The Id-specific CD4+ T cells recognize a CD3 epitope on the $\lambda^{315}$ light chain of M315, when presented on I-E\(^{k}\) MHC class II molecules (7, 21). Id-specific TCR-Tg mice (7), even on a SCID background (15), are resistant to subcutaneous challenge with MOPC315. Upon tumor challenge, such mice fail to develop palpable tumors, and myeloma protein is only transiently detectable, leading to the assumption that Id-specific CD4+ immune responses cause a complete and lasting rejection of tumor cells (15).

Here, longitudinal monitoring of myeloma cells in Id-specific TCR-Tg SCID mice revealed that a minute population of residual tumor cells remained in a dormant state for a prolonged period in TCRTg SCID mice (15). Immunoaffinity chromatography and metabolic labeling for the L chain (FLC), while the production of complete myeloma protein was unchanged. Reduction of FLCs was causally related to tumor escape.

**Materials and Methods**

**Cells and reagents**

MOPC315 (IgA, $\lambda^{315}$) is a BALB/c plasmacytoma obtained from the ATCC. mCherry-labeled MOPC315 cells were generated as previously described (22). The Ig nonproducing variant MOPC315.36, and MOPC315.37, which retains FLCs intracellularly due to a point mutation (Ala\(^{15}\)→Arg\(^{15}\)) in the L chain have been previously described (18). The MOPC315.37$\alpha^{315}$ variant was generated by stable transfection of MOPC315.37 cells with a pLNOH2 expression vector (23) containing a sequence encoding the H chain (V_H for the mutated L chain (24). When presented on I-E\(^{k}\) MHC, MOPC315, samples were passed through a column to remove cell debris, and the concentration was adjusted to $10^{5}$ cells/mL.

**Mice, tumor challenge, and tissue preparation**

Id-specific T-cell receptor transgenic (TCR-Tg) mice were generated from (CBA/Br × C57Bl6/Lia)F2 embryos, backcrossed for 10 generations to BALB/c (21), and crossed onto a BALB/c SCID background (15). For the current study, TCR-Tg SCID mice or SCID littersmates on BALB/c background were bred in a (non-transgenic SCID × heterozygous TCR-Tg SCID) fashion. Mice used in experiments were 4- to 6-week-old females weighing 16 to 25 g. Mice were injected subcutaneously with $1.6 \times 10^{5}$ tumor cells in 100 μL PBS or in 100 μL Matrigel (BD Biosciences). Tumor growth was followed using the IVIS200 Imaging System (Caliper LifeSciences). Mice were euthanized when tumor diameter reached >15 mm. Bromodeoxyuridine (BrdUrd) incorporation assays were performed as previously described (16). Preparation of cells for flow cytometry was performed as previously described (19). The sources of antibodies used are given in Supplementary Table S1.

**Real-time PCR**

Total RNA was extracted using the RNeasy Mini Kit (Qiagen), and cDNA generated using the First Strand cDNA Synthesis Kit (Life Technologies) with oligo-(dT) primers. RT-PCR was performed using TaqMan Universal PCR Master Mix on an ABI-Prism 7000 Sequence Detection System (Applied Biosystems) using predesigned FAM/TAMRA primer/probe sets (IDT Technologies). Results were quantified using ΔΔCt method with normalization against TATA-box binding protein (Tbp).

**In vitro T-cell proliferation and tumor cell growth inhibition assays**

Matrigel-infiltrating CD11b+ cells were isolated using CD11b MicroBeads (Miltenyi Biotech). Sorted CD11b+ cells (>95% pure) were irradiated (2,000 rad) before utilization in T-cell proliferation assays. Splenocytes isolated from SCID mice or tumor-derived CD11b+ cells were irradiated (2,000 rad) and utilized as stimulators (2 × 10^6 cells/well). Responders (2 × 10^6 cells/well) were short-term cultured Th1-polarized lymphocytes from TCR-Tg SCID mice, prepared as previously described (25). Supernant collected from 24-hour culture of 1 × 10^6 irradiated tumor cells was used as an antigen source. An optimal stimulatory concentration of synthetic 91-101 Id ($\lambda^{315}$) peptide (4 μg/mL) was added to the positive controls. M315, $\lambda^{315}$, and $\lambda^{7952}$ for use in T-cell proliferation assays were purified as previously described (26). For tumor growth inhibition assays, 1.5 × 10^5 tumor cells were cocultivated with 1.5 × 10^5 irradiated CD11b+ cells or splenocytes and 5 × 10^5 Id-specific T cells prepared as described above. Cultures were pulsed with [3H] thymidine (Montebello Diagnostics) after 24 hours, harvested 48 hours later, and counted using a TopCount NXT microplate counter (PerkinElmer).

**Immunoaffinity chromatography and metabolic labeling**

For the separation of complete M315 and free $\lambda^{315}$ light chains from MOPC315, samples were passed through a column.
containing sepharose-conjugated anti-Id Ab2-1.4 mAb, which requires association of the $\lambda_2^{115}$ with $H^{115}$ in a complete ($H+L$) M315 molecule for binding (27). Thus, free $\lambda_2^{115}$ L chain passes through the column. To obtain FLC/M315 ($H+L$) ratios, pre-/post-adsorption samples were analyzed in parallel by ELISA, as previously described (18). For high-throughput screening, cell culture supernatants were centrifuged in 96-well filter plates containing a 100 kDa cut-off membrane (AcroPrep Advance, Pall Corporation) and the flow-through collected for ELISA analysis as described above. Metabolic labeling of tumor cells was performed as previously described (28). Briefly, cells were washed in labeling medium (methionine-free RPMI1640 + 10% FCS and 0.5 mg/L methionine) and pulsed for 1 hour in labeling medium containing $350 \mu$Ci/mL $\text{L}^{-135}$S)-methionine (Hartmann Analytic GmbH). 

Nonreduced lysates and supernatants were immunoprecipitated for 12 hours at 4°C using sepharose-conjugated mAb specific for the C-domain of $\lambda_2$ (2B6). Bound protein was eluted using 0.1 mol/L Tris-citrate buffer and separated on 10% SDS-PAGE gels. Gels were soaked in 0.5 mol/L sodium salicylate (Sigma Aldrich) and developed using Amersham Hyperfilm MP (GE Healthcare).

**Mass spectrometry analysis and protein identification**

Affinity-purified M315 in solution was digested with trypsin before mass spectrometry analysis, and data were acquired as previously described (29). Acquired mass spectrometry data were submitted to de novo sequencing using PEAKS, using trypsin with no proline restriction as enzyme. Only peptide sequences with more than 85% average coverage certainty were submitted to BLAST analysis using the antibody sequence previously obtained by nucleotide sequencing of MOPC315 using the PEAKS DB option.

**Statistical analysis**

The Mann–Whitney U test was used for statistical analysis unless stated otherwise. For tumor challenge experiments, differences in survival were analyzed using the log-rank test. Statistical analysis was performed using Prism 5.0 software (GraphPad Software). $P < 0.05$ was considered statistically significant.

**Results**

Myeloma cells subject to CD4+ T-cell immune responses undergo immunoediting with eventual tumor escape

Utilizing fluorescently labeled MOPC315 cells, we monitored the fate of injected tumor cells by in vitro imaging. In accordance with previous reports (15), tumor-challenged SCID mice developed rapidly expanding tumors within 2 to 3 weeks after challenge (Fig. 1A). In contrast, the TCR-Tg SCID mice (hereafter referred to as TCR-Tg) showed a rapid decrease in fluorescent signal intensity. Nonetheless, by long-time monitoring, we observed that the TCR-Tg mice retained a weak but detectable fluorescent signal at the injection site (Fig. 1B and C), even though serum myeloma protein concentration was below the detection limit (Fig. 1D). Eventually, palpable tumors developed after a mean observation time of 12 ± 4 weeks (Fig. 1A). Concomitant with tumor development, M315 myeloma protein became detectable at levels comparable with that of similarly sized tumors in SCID mice (Fig. 1D).

Depletion of CD4+ cells in TCR-Tg mice harboring dormant tumor cells resulted in rapid tumor escape, confirming the importance of continued presence of Id-specific CD4+ T cells for immuno-surveillance (Supplementary Fig. S1A). Consistent with the recurrence of serum myeloma protein upon tumor escape, in vitro secretion of M315 by escape tumor cells (MOPC315.E) was unchanged compared with the parental MOPC315 cell line (data not shown), and peptide sequencing of the M315 L chain gene of MOPC315.E cells revealed no mutational changes within the antigen coding sequence (data not shown). Moreover, M315 isolated from cultures of parental or escape tumor cells showed comparable ability to stimulate proliferation of Id-specific T cells (Supplementary Fig. S1B).

To further identify the mechanisms of immuno-evasion, tumor cells were isolated from escape tumors, cloned, and re-injected into Id-specific TCR-Tg mice after 1 to 2 weeks of culture. A complete loss of immunoprotection against such cells was consistently observed (Fig. 1E).

**Tumor escape is caused by impaired intratumoral presentation of tumor-specific antigen**

On the basis of preserved secretion of the tumor-specific myeloma protein by the escape tumor cells, we hypothesized that immuno-evasion was caused by functional disturbances in the tumor-specific T-cell response. Arguing against this possibility, draining lymph nodes (DLN) from TCR-Tg mice harboring escape tumors still contained Id-specific T cells that proliferated upon in vitro restimulation at increased levels compared with naïve TCR-Tg T cells (Fig. 2A).

When syngeneic TCR-Tg mice were injected with escape tumor cells (MOPC315.E), a decreased Id-specific T-cell proliferation (Fig. 2B) and IFN-€/C16 expression (Fig. 2C) was observed in DLNs, even though Th1 polarization (Th1/Th17) was maintained (Fig. 2D). There was a corresponding decrease in the number of tumor-infiltrating Id-specific T cells (Fig. 2E). On the basis of the reduced numbers of responding T cells in DLN and tumors of MOPC315.E-challenged mice, it seemed possible that tumor escape could be caused by impaired local antigen presentation on tumor-infiltrating APCs. It was previously demonstrated that the bulk of tumor-infiltrating APCs in our model is formed by CD11b+Ly6C+ M1-like macrophages (19) associated with cytotoxic M1-like macrophage characteristics (16). To assay the antigen-presenting ability of these cells, CD11b+ cells were isolated from tumors of SCID mice challenged with MOPC315 or MOPC315.E cells, and used in in vitro T-cell proliferation assays. CD11b+ cells derived from escape tumors had an impaired ability to induce T-cell proliferation, but stimulatory capacity was restored upon addition of Id peptide (Fig. 3A), suggestive of reduced intratumoral antigen availability. Consistent with this, supernatants from escape tumor cells had a dramatically impaired ability to induce proliferation of Id-specific T cells in the presence of APC (Fig. 3B). This loss of T-cell proliferation could be reversed by reconstitution with 50% MOPC315-conditioned medium or by addition of Id peptide (Fig. 3B), suggesting that impaired T-cell activation was caused by inefficient antigen presentation in escape tumors.

Inflammation (19) associated with cytotoxic M1-like macrophage activation (16) has been implicated as a key mediator of successful tumor immuno-surveillance. The number of Matrigel-infiltrating CD11b+ cells was similar in mice challenged with MOPC315 and MOPC315.E cells (Fig. 3C), but CD11b+ cells from escape tumors were poorly activated, as evidenced by decreased expression of activation markers MHC class II and CD86 (Fig. 3D and data not shown).
Figure 1.
Tumor escape from CD4⁺ T-cell immunoprotection. A, survival of Id-specific TCR-Tg SCID (n = 45; hereafter referred to as TCR-Tg) and SCID mice (n = 8) challenged with 1.6 × 10⁵ MOPC315 cells. B and C, bioluminescence imaging of tumor development. Quantitative data are in B and representative examples in C. Mice were challenged subcutaneously with 1.6 × 10⁵ MOPC315-mCherry cells. White arrows (C) in top left panel indicate the injection site. Lanes 1 and 4 show SCID mice; lanes 2, 3, and 5 show TCR-Tg mice. Color scale was set at a radiance (photons/second/cm²/steradian/μW/cm²) of 6.7 × 10⁸–1.6 × 10⁹. D, serum M315 levels in TCR-Tg mice. Timepoint “Escape” represents the time of tumor escape, ranging from 10 to 20 weeks after challenge. Dotted line (n.d.) indicates detection threshold. The graph shows pooled data from two independent experiments (n = 10 mice). E, survival of TCR-Tg and SCID mice injected with MOPC315 or MOPC315.E cells (1.6 × 10⁵). Results are pooled from three independent experiments with MOPC315 or MOPC315.E cells derived from three different primary tumors established in TCR-Tg mice (n = 6–8 per treatment group). Bars, mean ± SD.
Id-specific CD4⁺ T-cell proliferation is dependent on secretion of free immunoglobulin light chains

On the basis of the above results, we reasoned that loss of T-cell activation might be dependent on the tumor antigen being present in another form than complete M315 Ig.

MOPC315 cells, similar to MM cells in general, are known to produce excess amounts of Ig L chains detectable as FLC. We therefore assayed the secretion of FLCs by MOPC315 and MOPC315.E cells into cell culture supernatants. A significant reduction in free λ2 L chain secretion was found for all escape tumors (Fig. 4A, P < 0.001), while complete (H+L) Ig was present at levels comparable with that of parental cells (Fig. 4A). Assays of clones derived by limiting dilution revealed homogenous FLC secretion by the parental MOPC315 cells, and a relatively uniform decrease in FLC secretion by escape tumor clones obtained by limiting dilution from escape tumors (Fig. 4B). Western blots of tumor cell lysates revealed a selective decrease in the amount of FLCs secreted (Fig. 4C). Correspondingly, mRNA expression of the λ2 light chain was significantly decreased in escape tumor clones (Fig. 4D), with a significant correlation between λ2 mRNA expression level and the amount of FLCs secreted (Supplementary Fig. S2A). In contrast, IgA heavy chain mRNA levels remained unchanged (Fig. 4D). Metabolic labeling experiments revealed similar kinetics of Ig secretion by parental and escape tumor cells (Fig. 4E and Supplementary Fig. S2B), with no evidence of L chain retention in escape tumor cells (Fig. 4E). L chains appeared in the culture supernatant primarily in monomeric form, in accordance with previous reports (30), but was present at significantly lower levels in cultures of escape tumor cells than in parental cells (Fig. 4A). In summary, these results demonstrate a selective decrease in light chain transcription, reflected in impaired secretion of FLCs, whereas synthesis remains sufficient to allow secretion of complete L+H Ig.

Given the selective reduction in FLCs in escape tumors, with secretion of complete (H+L) M315 myeloma protein remaining essentially unchanged, we reasoned that the two different quaternary forms of the tumor-specific antigen could differ in their ability to stimulate tumor-specific CD4⁺ T cells. In accordance with a previous report (20), in vitro T-cell proliferation assays demonstrated that isolated λ2 FLCs had a 100-fold greater ability to induce T-cell proliferation compared with equimolar amounts of complete M315 (Fig. 4F).

Id-driven CD4⁺ T-cell immunoprotection against MOPC315 myeloma cells is dependent on secretion of FLCs

Overexpression of λ2 ³⁺ L chain under the control of a viral promoter in escape tumor cells increased light chain synthesis (Fig. 5A) and restored immunoprotection (Fig. 5B). In contrast,
overexpression of the mutant λ2315 light chain, which differs from λ2T952 in the amino acid composition within the idiotope (aa 94-99) in MOPC315.E cells, failed to reverse the escape phenotype (Fig. 5B). These results conclusively demonstrate that tumor escape is caused by impaired secretion of λ2315 L chain.

To further confirm the importance of FLC secretion for immunoprotection, we utilized the previously described MOPC315.37-variant cell line. These cells produce no heavy (H) chain, and contain a point mutation within the L chain variable (V) region (Ala15→Arg15) that causes retention of FLCs within the ER, preventing its secretion (31, 32). We found that by transfecting MOPC315.37 cells with a plasmid expressing the IgA M315 H chain, secretion of a complete H+L Ig was obtained even though FLC secretion was still absent (presumably, upon association with an H chain, the ER retention signal on the mutated L chain is masked). This ensures secretion of only the complete M315-like molecules by the resultant MOPC315.37α315 cell line (Fig. 5C).

Tumor challenge experiments in TCR-Tg mice revealed that injection of MOPC315.37 cells led to rapid tumor development in both SCID and TCR-Tg mice at day +8 following challenge with Matrigel-embedded MOPC315 or MOPC315.E cells. Results represent pooled data from two independent experiments (n = 12 per treatment group). Bars, mean ± SD.

Loss of λ2 FLC secretion is a consequence of T-cell–mediated immunoediting

To establish whether loss of FLC secretion occurs as the result of in vivo immunoediting rather than through selection of preexisting clones, MOPC315 clones were derived by limiting dilution. Tumor challenge experiments were performed using clones with high levels of FLC secretion. Clonally derived tumor cells were subject to immunoediting, causing tumor escape within the same timeframe as for the heterogeneous parental MOPC315 population (Fig. 5E), and escape tumor cells displayed similarly suppressed L chain transcription and impaired FLC secretion (Fig. 5F).

A possible explanation for these results could be that variants expressing low levels of FLCs might arise during in vitro expansion of cloned cells, required to obtain sufficient number of tumor cells for injection. To test this, cells from the injection inoculum were recloned and assayed for FLC secretion. Light chain secretion in the clonally derived cells was found to be homogenous, with no FLC loss variants identified among 900 screened cells (Supplementary Fig. S2C), essentially excluding the possibility of FLC loss occurring during the in vitro expansion phase before tumor challenge.

Discussion

The current results demonstrate that an initially successful CD4+ T-cell response against Id-secreting myeloma cells is eventually overcome by residual tumor cells that persist in a dormant state for several months. The escape tumor cells retain their ability to resist T-cell–mediated killing upon rechallenge into Id-specific TCR transgenic mice, even after cloning and prolonged culture, suggesting that the underlying mechanisms represent stable changes intrinsic to the tumor cells.
Further characterization of escape tumor cells revealed that the antigen responsible for T-cell activation is not, as previously assumed, the complete M315 Ig molecule, but rather a surplus of L chains that are secreted in a free form, not assembled with H chains, by the myeloma cells. Tumor escape was associated with a dramatic reduction in secretion of FLCs, despite continued...
secretion of the complete myeloma protein. Importantly, the L chain assembled in the H+L myeloma protein still expressed the tumor specific Id-epitope, as no mutations were found, and as myeloma protein from escape tumors were fully stimulatory to Id-specific CD4⁺ T cells. Experimental reintroduction of FLC secretion in escape tumor cells resulted in prompt rejection by Id-specific T cells, providing a causal link between the presence of FLCs and successful tumor rejection. Further evidence was obtained by H chain transfection of a MOPC315 tumor cell variant that normally retains a mutated L chain. Again, secretion of FLCs was needed for rejection to occur. Taken together, these findings demonstrate that the presence of the tumor antigen in the form of a protein complex (assembled M315 IgA) impairs the accessibility of the epitope for presentation on host APCs compared with free, noncomplexed L chains. Thus, a change in the abundance of one protein conformation allows tumor escape from CD4⁺ T cells even though the peptide sequence recognized by T cells is unchanged and remains available in a complexed form. These observations most likely relate to differences in antigen processing of complete Ig and FLCs within the APCs. Accordingly, we demonstrate that FLCs are 100 times more efficient than complete (H+L) Ig at stimulating Id-specific T cells. This finding is in agreement with previous experiments demonstrating that complete (H+L) M315 in either native or reduced/alkylated form, as well as Fab, fed to spleen APCs were all poorly stimulatory to cloned Id-specific CD4⁺ T cells. In contrast, "preprocessed" fragments such as free λ2 chains, VL and FV fragments were all highly stimulatory (20). It could thus be speculated that properties of the assembled Fab region of the Ig molecule might prevent complete antigen processing, possibly due to effects of the noncovalent...
interaction between Fd fragments (VH+CH1) and to the L chain. This would limit availability of short V region sequences for binding to MHC class II molecules in endosomes, resulting in diminished Id presentation. Previous experiments have indicated that processing of complete Ig in macrophages results in accumulation of Fab-like molecules, suggestive of a relative resistance of Ig to proteolytic enzymes (33). The issue of differences in processing of various forms of Ig light chains, either in free form or associated with H-chains, in APCs is the subject of ongoing investigations.

The influence of conformation of processing of proteins is not unique to Ig but may be extended to such as insulin (34), hemoglobin (35) and lysozyme (36). Modulation of the form of the antigen produced by the tumor cells may constitute an under-appreciated mode of immunoediting. Hence, our findings of altered FLC availability might also be relevant for other tumor antigens that form complexes. This sort of analysis requires insight into the forms in which the antigen is present, its secretory status and, most likely, the mode of T-cell-mediated killing. Further evaluation of this issue is complicated by the number of presently available models of CD4+ T-cell responses against bona fide tumor antigens, as recently reviewed (37). Nonetheless, our results underscore the importance of considering tertiary and quaternary protein structure when looking at T-cell responses against such antigens.

MHC class II expression is normally limited to professional APCs, although IFNγ has been shown to induce surface expression of MHC class II in some types of malignant cells. Similar to most myeloma cells, MOPC315 cells are MHC class II negative, even in the presence of IFNγ (16, 18). Hence, recognition of Id by CD4+ T cells is critically dependent on display on host APCs (18). Other groups have similarly shown a dependence of anti-tumor CD4+ T-cell responses on host MHC II expression (14), even when tumor cells themselves are MHC II+ (8, 9). Thus, display of tumor antigen–derived peptides on host APC may be universally required for tumor protective CD4+ T-cell responses. It thus seems possible that malignant cells may circumvent CD4+ T-cell responses by changing the expression of tumor-specific antigens so that display on host APCs is impaired. The current data exemplify this type of evasion strategy, and further show that such effects can be obtained by restricting antigen availability to a poorly immunogenic multiprotein complex, most likely influencing antigen uptake and processing within the APCs.

Concerning the mechanism underlying the escape phenotype, the loss of FLC secretion was associated with impaired mRNA expression levels, and a decreased abundance of FLCs in cell lysates. The levels of complete M315 secretion by escape tumor cells were not significantly decreased compared with parental cells, although some heterogeneity in the levels of secreted M315 was observed among the escape tumor clones. The basis for the resulting balanced synthesis of H and L chains in escape tumor cells is not known. Previous reports have demonstrated that unbound H chains have cytotoxic effects (38, 39), which provides a selective pressure against excess synthesis of H chains. At the same time, the high protein turnover in myeloma cells imposes a considerable endoplasmic reticulum stress (reviewed in ref. 40), which increases susceptibility to cell death by added stressors such as reactive oxygen species. Thus, the balance of L and H chain synthesis might represent a tradeoff between a need to avoid heavy chain-mediated cytotoxicity (41) and beneficial effects of reduced L chain availability in the face of the Id-specific T-cell response. Whereas outgrowth of tumor cells secreting only L chains is observed in a subset of myeloma patients during disease progression (so-called "FLC escape"; refs. 42, 43), the occurrence of loss of FLC secretion has not been reported. It should be underscored that this phenomenon of "FLC escape" represents an entity with no obvious relation to the current findings. Although CD4+ T-cell reactivity against myeloma cells in patients receiving conventional treatment cannot be excluded, selective outgrowth of tumor cells with impaired FLC secretion seems unlikely to occur in the absence of immunotherapeutic interventions targeting Id. Nonetheless, it seems plausible that the mechanisms driving the loss of FLC secretion could be related to tumor cell adaptation to other kinds of cellular stress, for example, development of resistance to cytotoxic drugs. This issue is being addressed through ongoing experiments.

It should also be emphasized that the escape phenotype appears not to be due to preexisting variants within the tumor cell inoculum. Clonally derived MOPC315 cells with stable expression of both H+L and FLC secretion consistently underwent downregulation of FLC secretion in the presence of an ongoing Id-specific CD4+ T-cell response. These results argue for an acquisition of the escape phenotype in vivo as a consequence of Th1/M1-mediated immune pressure on tumor cells.

The current study identifies a novel mechanism of tumor immuno evasion from an anti-tumor CD4+ T-cell response. Immune escape develops through a modulation of secretion of a natural tumor-specific antigen, limiting availability of the antigen in the conformation that is required for efficient display on tumor-infiltrating APCs. Consequently, T-cell activation and induction of cytotoxic macrophages is dampened. This highlights the importance of efficient local antigen display within the tumor microenvironment for potent CD4+ T-cell–mediated immunotherapy of MHCII- tumor cells, and extends the framework for investigations into mechanisms of tumor immunoediting.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Tumor Escape from CD4+ T Cells

References


Tumors Escape CD4+ T-cell –Mediated Immunosurveillance by Impairing the Ability of Infiltrating Macrophages to Indirectly Present Tumor Antigens

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