*Commentary*

# CD4 T Cells and Their Role in Antitumor Immune Responses

By René E.M. Toes,*‡ Ferry Ossendorp,* Rienk Offringa,* and Cornelis J.M. Melief*

*From the *Department of Immunohematology and Blood Bank, Leiden University Medical Center, 2333 AZ Leiden, The Netherlands; and ‡Abteilung Immunologie, Institut für Zellbiologie, D-72076 Tübingen, Germany*

The specificity and power of the cellular arm of the immune system may provide new therapeutic approaches to cancer. With the assumption that T cells might be able to recognize and eliminate cancer cells with the same efficiency as virus-infected cells, investigators have searched many years for ways to trigger or amplify the patient's inadequate immune response to tumors. Much attention has been given to the role of $CD8^+$ CTLs because most tumors are MHC class I positive, but negative for MHC class II. Moreover, $CD8^+$ CTLs are able to lyse tumor cells directly upon recognition of peptide–MHC class I complexes expressed by the tumor, and their ability to eradicate large tumor masses in vivo has been demonstrated. The focus in cancer immunology on $CD8^+$ T cell responses is also exemplified by an increasing list of tumor antigens identified by tumor-reactive $CD8^+$ CTLs. $CD4^+$ Th cells have received far less attention, which is remarkable given the pivotal role of these cells in regulating most antigen-specific immune responses. Until now, only a few Th epitopes derived from human tumor antigens recognized by $CD4^+$ Th cells have been identified (1, 2). Three studies published in this issue describe the identification of melanoma antigens that are recognized by $CD4^+$ T cells in the context of MHC class II molecules (3–5). Charting the Th response against human melanoma as well as other tumors is important for the development of optimal anticancer vaccines and for the design of other T cell–related therapeutic modalities in cancer.

*Lessons Learned from Mouse Tumor Models.* Studies using adoptively transferred purified T cell subsets or in vivo depletion studies have firmly established an important role for tumor-specific $CD8^+$ CTLs in antitumor immunity (for a review, see reference 6). By comparing the relative contribution of $CD4^+$ and $CD8^+$ T cells to the overall immune response, it was shown that activated adoptively transferred $CD8^+$ T cells alone are as effective as adoptively transferred $CD4^+$ and $CD8^+$ T cells, provided that IL-2 is given simultaneously (7–9). Nonetheless, a critical contribution by tumor-specific $CD4^+$ Th cells in the development of an effective antitumor tumor response was consistently found in several murine tumor models (10–13). $CD4^+$ T cells are likely to play a diversified role in antitumor immunity that includes several distinct antitumor effector functions. The role of $CD4^+$ T cells in priming of CTLs is well documented (14), explaining why activated CTLs, but not naive CTLs, can mediate potent antitumor effects in the absence of $CD4^+$ T cells. Analysis of the participation of individual T cell populations in the elimination of the Friend murine leukemia virus (MuLV)–induced tumor FBL-3, revealed that tumor-specific $CD4^+$ T cells can also exert their effect independently of $CD8^+$ CTLs. Adoptive transfer studies showed that both the noncytolytic $CD4^+$ subset as well as the cytolytic $CD8^+$ subset were individually capable of eradication of disseminated leukemia in tumor-bearing mice (for a review, see reference 15). Thus, although the tumor-specific MHC class II–restricted $CD4^+$ T cells are not able to recognize this MHC class II–negative tumor directly, they are able to control tumor growth via a mechanism that does not require CTLs (16). More recently, it was shown that not only adoptive transfer of $CD4^+$ T cells, but also vaccination with an MuLV-derived Th epitope (but not a control Th peptide) induced protection against a subsequent challenge with MHC class II–negative, virus-induced tumor cells (17). In this case, the protection induced was dependent on both $CD4^+$ and $CD8^+$ T cells, as depletion of either subset at the time of tumor challenge abrogated the ability to control tumor outgrowth. Simultaneous vaccination with a tumor-specific CTL epitope and the tumor-specific Th epitope, rather than an unrelated Th epitope, resulted in strong synergistic protection. Taken together, these findings illustrate the relevance of identifying and using tumor-specific Th epitopes even in the case of MHC class II–negative tumors, and emphasize the importance of activating both tumor-specific $CD8^+$ and $CD4^+$ cells to establish optimal immunity to cancer.

*Orchestration of the Antitumor Immune Response by $CD4^+$ T Cells.* As evident from the experience in the MuLV tumor models, tumor-specific $CD4^+$ T cells can mediate several functions influencing the outcome of tumor-specific immunity. Numerous studies have focussed on the role $CD4^+$ T cells play in delivery of help for priming of tumor-specific CTLs, resulting in important mechanistic insights into this event. Accumulating evidence indicates that for induction of MHC class I–restricted tumor-specific immunity, cross-presentation of antigens that have been captured by professional APCs plays a dominant role (18–21).

Dissection of the cellular interactions involved in CTL priming revealed that Th cells must recognize antigen on the same APC that cross-presents the CTL epitope (22). These findings explain the requirement for epitope linkage between Th cell epitopes and CTL epitopes important for induction of CTL responses (23), and could clarify the view that help for CTLs is delivered through the release of soluble factors such as IL-2 produced by Th cells in the proximity of CTLs. Recently, however, it was shown that T cell help for CTLs is critically dependent on interaction between CD40L expressed by Th cells and CD40 expressed by APCs (24, 25). Indeed, a central role for CD40–CD40L interactions in the generation of protective T cell–mediated tumor immunity has been demonstrated (26, 27). These interactions most likely empower the APCs to prime CTLs, since help for CTL priming can be bypassed by activation of dendritic cells (DCs) through CD40 (28). Several lines of evidence indicate that CD40 signaling is part of an important pathway in T cell–dependent APC activation. Recombinant soluble CD40L stimulates human monocytes to release proinflammatory cytokines (29), whereas ligation of CD40 on DCs or interactions between DCs and $CD4^+$ T cells triggers the production of IL-12. In the latter case, IL-12 production by DCs was inhibited by blockade of CD40L on the $CD4^+$ T cell (30). Moreover, CD40 ligation is a potent stimulus to upregulate the expression of intercellular adhesion molecule 1 (ICAM-1), CD80, and CD86 molecules (31, 32). Because CD40-induced activation of professional APCs results in the expression of costimulatory molecules important for CTL priming, this activation is likely to play an important role in the delivery of T help to CTLs. In this model, the APC that cross-presents antigen to both antigen-specific Th cells and CTLs acts as an intermediary for the delivery of help to CTLs.

Appreciation of the fundamental role of the APC activation state to tune the outcome of T cell responsiveness helps to explain why CTL responses against tumors, including those induced by noninflammatory persistent tumor viruses such as MuLV (and likely human papillomavirus and Epstein-Barr virus), are dependent on T cell help, whereas CTL responsiveness against acute disease-causing cytopathic viruses such as influenza virus is without a clear need for $CD4^+$ Th activity (33). The difference between these two situations appears to reside in the fact that influenza virus can directly infect and activate DCs to a phenotype conducive to CTL activation in a $CD4^+$ T cell–independent fashion (28). However, under noninflammatory conditions, such as in many allograft situations and in most cancers, CTL responses are much more Th cell dependent because DCs need to be activated first by specific $CD4^+$ T cells before they trigger T killer responses.

Besides their intimate involvement in priming tumor-specific CTLs, $CD4^+$ Th cells participate in additional effector functions. Evidence that these other Th cell–dependent effector mechanisms play an important role in the host defence against tumors came from studies in the MuLV system in which adoptively transferred tumor-specific $CD4^+$ T cells are implicated in the activation of tumoricidal macrophages involved in tumor clearance (15). More recently, it was demonstrated in a model involving vaccination with irradiated tumor cells, transduced to secrete GM-CSF, that cytokines produced by $CD4^+$ T cells belonging to the Th1 or Th2 lineage can recruit and activate macrophages and eosinophils, respectively (13). Protection against tumor challenge was strongly associated with the presence of eosinophils at the tumor challenge site as well as the production of oxygen radicals by tumoricidal macrophages, since genetically modified mice disabled to produce these radicals were severely hampered in their ability to resist tumor challenge. A significant fraction of CD8 knockout, but not CD4 knockout animals, were able to successfully resist tumor challenge, indicating that the observed effects relied on $CD4^+$ T cell–mediated effector mechanisms.

As in the MuLV system, the tumor described above did not express MHC class II molecules, emphasizing the notion that induction or propagation of $CD4^+$ T cell–mediated immunity can be successfully applied to counteract tumors that lack or lose expression of MHC molecules.

*Induction of Tumor-specific T Cell Tolerance*. The studies described above, together with the identification of new tumor antigens recognized by $CD4^+$ T cells, bring fresh encouragement to the development of anticancer immune intervention schemes. However, manipulation of the immune response to tumors in tumor-bearing hosts might be actively frustrated by the tumor itself, as it has been reported that tumors can induce tumor-specific $CD4^+$ T cell nonresponsiveness (34). The mechanism of tolerization is, as yet, not clear, but it might mimic many aspects described for tolerance induction to peripheral tissue antigens. Peripheral tolerance induction of both antigen-specific $CD4^+$ and $CD8^+$ T cells to antigens expressed outside the lymphoid system has been described in several models (35–37). In these cases, tolerance is mediated by cross-presentation of the antigen on bone marrow–derived APCs (36, 37). As development and growth of tumors is initially not accompanied by inflammatory stimuli or stress to the immune system, antigen derived from the tumor might be shunted in the same cross-tolerizing pathway as reported for peripheral tissue antigens. In this way tumors, as close mimics of the normal tissue from which they are derived, might shrewdly use the T cell tolerizing state of certain bone marrow–derived APCs that normally guarantee tissue tolerance. This tolerization of both helpers and killers might hamper immune intervention schemes that are based on the induction or propagation of the T cell immune system in tumor-bearing hosts. Knowledge about the epitopes recognized by human tumor-specific $CD4^+$ and $CD8^+$ T cells will be instrumental to study whether such a scenario could explain why certain tumors—for instance, melanoma—are able to grow, despite the expression of potentially highly immunogenic tumor antigens.

In summary, tumor-specific $CD4^+$ Th cells can orchestrate several effector functions that can cooperate in an effective antitumor response. Knowledge of the antigens and peptides recognized by human $CD4^+$ T cells is of crucial importance for a better understanding of the behavior and

role these cells play in the immune response to human tumors, as well as for optimal use of the Th arm of the immune system in the development of new anticancer vaccine modalities. The studies published in this issue describing new MHC class II–restricted melanoma antigens and new methods to identify tumor antigens recognized by $CD4^+$ T cells point to the vital role $CD4^+$ T cells have in immune attack directed against human tumors, and will be of great benefit in optimizing tumor immunotherapy if the rules of the murine models apply to the situation in cancer patients.

We thank Dr. H.G. Rammensee and Dr. T.H.M. Ottenhoff for critical reading of the manuscript.

R.E.M. Toes is a fellow of the Royal Netherlands Academy of Arts and Sciences.

Address correspondence to René E.M. Toes, Department of Immunohematology and Blood Bank, Leiden University Medical Center, P.O. Box 9600, 2333 AZ Leiden, The Netherlands. Phone: 31-71-5263800; Fax: 31-71-5216751; E-mail: r.e.m.toes@immunohematology.medfac.leidenuniv.nl

*Received for publication 13 January 1999.*

## References

1. Halder, T., G. Pawelec, A.F. Kirkin, J. Zeuthen, H.E. Meyer, L. Kun, and H. Kalbacher. 1997. Isolation of novel HLA-DR restricted potential tumor-associated antigens from the melanoma cell line FM3. *Cancer Res.* 57:3238–3244.
2. Topalian, S.L., M.I. Gonzales, M. Parkhurst, Y.F. Li, S. Southwood, A. Sette, S.A. Rosenberg, and P.F. Robbins. 1996. Melanoma-specific $CD4^+$ T cells recognize nonmutated HLA-DR–restricted tyrosinase epitopes. *J. Exp. Med.* 183:1965–1971.
3. Manici, S., T. Sturniolo, M.A. Imro, J. Hammer, F. Sinigaglia, C. Noppen, G. Spagnoli, B. Mazzi, M. Bellone, P. Dellabona, and M.P. Protti. 1999. Melanoma cells present a MAGE-3 epitope to $CD4^+$ cytotoxic T cells in association with histocompatibility leukocyte antigen DR11. *J. Exp. Med.* 189:871–876.
4. Pieper, R., R.E. Christian, M.I. Gonzales, M.I. Nishimura, G. Gupta, R.E. Settlage, J. Shabanowitz, S.A. Rosenberg, D.F. Hunt, and S.L. Topalian. 1999. Biochemical identification of a mutated human melanoma antigen recognized by $CD4^+$ T cells. *J. Exp. Med.* 189:757–765.
5. Chaux, P., V. Vantomme, V. Stroobant, K. Thielemans, J. Corthals, R. Luiten, A.M.M. Eggermont, T. Boon, and P. van der Bruggen. 1999. Identification of MAGE-3 epitopes presented by HLA-DR molecules to $CD4^+$ T lymphocytes. *J. Exp. Med.* 189:767–777.
6. Melief, C.J.M. 1992. Tumor eradication by adoptive transfer of cytotoxic T lymphocytes. *Adv. Cancer Res.* 58:143–175.
7. Kast, W.M., A.M. Bronkhorst, L.P. de Waal, and C.J.M. Melief. 1986. Cooperation between cytotoxic and helper T lymphocytes in protection against lethal sendai virus infection. *J. Exp. Med.* 164:723–738.
8. Bear, H.D., B.M. Susskind, K.A. Close, and S.K. Barrett. 1988. Phenotype of syngeneic tumor-specific cytotoxic T lymphocytes and requirements for their in vivo generation from tumor-bearing host and immune spleens. *Cancer Res.* 48:1422–1427.
9. Kast, W.M., R. Offringa, P.J. Peters, A.C. Voordouw, R.H. Meloen, A.J. Van der Eb, and C.J.M. Melief. 1989. Eradication of adenovirus E1-induced tumors by E1a-specific cytotoxic T lymphocytes. *Cell.* 59:603–615.
10. Greenberg, P.D., M.A. Cheever, and A. Fefer. 1981. Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic $Lyt1^+2^-$ lymphocytes. *J. Exp. Med.* 154: 952–963.
11. Schild, H., B. Kyewski, P. Von Hoegen, and V. Schirrmacher. 1987. CD4+ helper T cells are required for resistance to highly metastatic murine tumor. *Eur. J. Immunol.* 17: 1863–1866.
12. Romerdahl, C.A., and M.L. Kripke. 1988. Role of helper T-lymphocytes in rejection of UV-induced murine skin cancers. *Cancer Res.* 48:2325–2328.
13. Hung, K., R. Hayashi, A. Lafond-Walker, C. Lowenstein, D. Pardoll, and H. Levitsky. 1998. The central role of $CD4^+$ T cells in the antitumor immune response. *J. Exp. Med.* 188: 2357–2368.
14. Keene, J., and J. Forman. 1982. Helper activity is required for the in vivo generation of cytotoxic T lymphocytes. *J. Exp. Med.* 155:768–782.
15. Greenberg, P.D. 1991. Adoptive T cell therapy of tumors: mechanisms operative in the recognition and elimination of tumor cells. *Adv. Immunol.* 49:281–355.
16. Greenberg, P.D., D.E. Kern, and M.A. Cheever. 1985. Therapy of disseminated murine leukemia with cyclophosphamide and immune $Lyt\text{-}1^+,2^-$ T cells. Tumor eradication does not require participation of cytotoxic T cells. *J. Exp. Med.* 161:1122–1134.
17. Ossendorp, F., E. Menged, M. Camps, R. Filius, and C.J.M. Melief. 1998. Specific T helper cell requirement for optimal induction of cytotoxic T lymphocytes against major histocompatibility complex class II–negative tumors. *J. Exp. Med.* 187:693–702.
18. Bevan, M.J. 1976. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. *J. Exp. Med.* 143: 1283–1288.
19. Seung, S., J.L. Urban, and H. Schreiber. 1993. A tumor escape variant that has lost one major histocompatibility complex class I restriction element induces specific $CD8^+$ T cells to an antigen that no longer serves as a target. *J. Exp. Med.*

178:933–940.
20. Huang, A.Y.C., P. Golumbek, M. Ahmadzadeh, E. Jaffee, D. Pardoll, and H. Levitsky. 1994. Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science*. 264:961–965.
21. Toes, R.E.M., R.J.J. Blom, E. van der Voort, R. Offringa, C.J.M. Melief, and W.M. Kast. 1996. Protective anti-tumor immunity induced by immunization with completely allogeneic tumor cells. *Cancer Res*. 56:3782–3783.
22. Bennett, S.R.M., F.R. Carbone, F. Karamalis, J.F.A.P. Miller, and W.R. Heath. 1997. Induction of a $CD8^+$ cytotoxic T lymphocyte response by cross-priming requires cognate $CD4^+$ T cell help. *J. Exp. Med*. 186:65–70.
23. Cassel, D., and J. Forman. 1988. Linked recognition of helper and cytotoxic antigenic determinants for the generation of cytotoxic T lymphocytes. *Ann. NY Acad. Sci.* 532:51–60.
24. Schoenberger, S.P., R.E.M. Toes, E.I.H. van der Voort, R. Offringa, and C.J.M. Melief. 1998. T help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature*. 393:480–483.
25. Bennet, S.R.M., F.R. Carbone, F. Karamalis, R.A. Flavell, J.F.A.P. Miller, and W.R. Heath. 1998. Help for cytotoxic T cell responses is mediated by CD40 signalling. *Nature*. 393: 478–480.
26. Mackey, M.F., J.R. Gunn, P.P. Ting, H. Kikutani, G. Dranoff, R.J. Noelle, and R.J. Barth, Jr. 1997. Protective immunity induced by tumor vaccines requires interactions between CD40 and its ligand, CD154. *Cancer Res*. 57:2569–2574.
27. Mackey, M.F., J.R. Gunn, C. Maliszewski, H. Kikutani, R.J. Noelle, and R.J. Barth, Jr. 1998. Dendritic cells require maturation via CD40 to generate protective antitumor immunity. *J. Immunol*. 161:2094–2098.
28. Ridge, J.P., F. Di Rosa, and P. Matzinger. 1998. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature*. 393:474–478.
29. Kiener, P.A., D.P. Moran, B.M. Rankin, A.F. Wahl, A. Aruffo, and D. Hollenbaugh. 1995. Stimulation with purified soluble CD40L induces proinflammatory responses in human monocytes. *J. Immunol*. 155:4917–4925.
30. Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kampgen, N. Romani, and G. Schuler. 1996. High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J. Exp. Med*. 184:741–746.
31. Cella, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T–T help via APC activation. *J. Exp. Med*. 184:747–752.
32. Shinde, S., Y. Wu, Y. Guo, Q. Niu, J. Xu, I.S. Grewal, R. Flavell, and Y. Liu. 1996. CD40L is important for induction of, but not response to, costimulatory activity. ICAM-1 as the second costimulatory molecule rapidly upregulated by CD40L. *J. Immunol*. 157:2764–2768.
33. Allan, W., Z. Tabi, A. Cleary, and P.C. Doherty. 1990. Cellular events in the lymph node and lung with influenza. Consequences of depleting CD4+ T cells. *J. Immunol*. 144:3980–3986.
34. Staveley-O'Carroll, K., E. Sotomayor, J. Montgomery, I. Borrello, L. Hwang, S. Fein, D. Pardoll, and H. Levitsky. 1998. Induction of antigen-specific T cell anergy: an early event in the course of tumor progression. *Proc. Natl. Acad. Sci. USA*. 95:1178–1183.
35. Foster, I., and I. Lieberam. 1996. Peripheral tolerance of CD4 T cells following local activation in adolescent mice. *Eur. J. Immunol*. 26:3194–3202.
36. Kurts, C., H. Kosaka, F.R. Carbone, J.F.A.P. Miller, and W.R. Heath. 1997. Class I–restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive $CD8^+$ T cells. *J. Exp. Med*. 186:239–245.
37. Adler, A.J., D.W. Marsh, G.S. Yochum, J.L. Guzzo, A. Nigam, W.G. Nelson, and D.W. Pardoll. 1998. $CD4^+$ T cell tolerance to parenchymal self-antigens requires presentation by bone-marrow–derived antigen-presenting cells. *J. Exp. Med*. 187:1555–1564.