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Comment on "Germinal Center Helper T Cells Are Dual Functional Regulatory Cells with Suppressive Activity to Conventional CD4 $^+$ T Cells"

Daniele Focosi and Mario Petrini

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LETTERS TO THE EDITOR

Comment on "Germinal Center Helper T Cells Are Dual Functional Regulatory Cells with Suppressive Activity to Conventional CD4$^+$ T Cells"

Marinova et al. (1) show that germinal center (GC) CD4$^+$CD57$^+$ T cells represent a novel population of regulatory T cells that express CTLA-4 and glucocorticoid-induced TNF[1] receptor but not FOXP3. Although they help GC B cells, these cells inhibit CD4$^+$CD57$^-$ memory/effector T cells via IL-10, TGF-β, and CD95-CD95L interaction. In 2005, Kim et al. (2) showed that CD4$^+$CD45RO$^+$CD45RA$^-$CD57$^+$CCR7$^-$CXCR5$^+$ T cells in the GC of human lymphoid tissues comprise the major T cell subset that helps GC B cells produce immunoglobulins, acting via CD40L.

However, contrary evidence exists. In 1995, Bouzahah et al. (3) showed that human tonsillar CD4$^+$CD57$^+$ T cells, which mainly occupy the light zones of the GC where few B cells divide, did not markedly enhance B cell proliferation, contrary to GC CD4$^+$CD57$^-$ T cells. In 1996, Andersson et al. (4) showed that the addition of exogenous IL-2 was required for induction of Ig production by CD4$^+$CD57$^+$ GC helper T cells in vitro, although these cells spontaneously rescued GC B cells from cell death.

In 2006, Rasheed et al. (5) showed that in GC T cells from human tonsils, differences in stimulatory activity and CXCL13 secretion were related only to the expression levels of the homeostatic chemokine receptor CXCR5 and the costimulatory molecule ICOS, indicating that CD57 does not serve as a surrogate marker for helper activity. Even if we agree that the morphological interrelations between CD4$^+$CD57$^+$ GC T cells and follicular dendritic cells (as first described in 1989 by Yuda et al. (6)) and the appearance of CD57$^+$ cells during the transition from the primary follicle to the GC can be misleading, Marinova et al. should have made clear to readers that they were investigating a cell population that lacks the optimal surrogate markers for GC B cell help.

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Response to Comment on "Germinal Center Helper T Cells Are Dual Functional Regulatory Cells with Suppressive Activity to Conventional CD4$^+$ T Cells"

It seems that Drs. Focosi and Petrini (1) classify T cells as helpers for B cells only when they are able to promote B cell proliferation. It is common knowledge that there are at least two different types of Th signals during T-dependent Ag-driven B cell activation and differentiation: 1) helper signals for the initial B cell activation, which mainly takes place in the T-B junction and initiates B cell proliferation; and 2) helper signals for B cell functional maturation, which mainly takes place in the germinal center (GC) and promotes B cell differentiation such as class switching and memory cell development. It has been generally accepted that GC helper T cells are critical for the survival of B cell clones that have a competitive advantage for limited Ags, leading to clonal selection and affinity maturation (2–4). Our work (5) and another publication (6) cited in the letter of Drs. Focosi and Petrini were about T helpers for the GC pathway of B cell differentiation, showing that CD4$^+$CD57$^+$CXCR5$^+$ T cells are the major helpers for B cell survival and functional maturation. The work of Bouzahzah and colleagues (7) showing enhancement of B cell proliferation by CD4$^+$CD57$^-$ T cells but not by CD4$^+$CD57$^+$ T cells was not contradictory to the notion that CD4$^+$CD57$^+$ T cells mainly provide helper signals for GC reaction. The work of Andersson and colleagues (8) studied CD57$^+$ T cells from peripheral blood, not GC T cells as quoted by Drs. Focosi and Petrini. It should be noted that the differentiation pathways and properties of most peripheral blood CD57$^+$ T cells are different from those of tonsil CD57$^+$ T cells. Nevertheless, the findings of a requirement for IL-2 and supporting B cell survival by peripheral blood CD57$^+$ T cells do not conflict with our results. Mixed results were obtained from the work of Rasheed and colleagues (9) that used total tonsil B cells instead of purified GC B cells. In addition, CXCL13 production is not exactly a good marker for Th function. The major sources of CXCL13 in the follicular area are stromal cells such as follicular dendritic cells. Therefore, the publications (7–9) referred by Drs. Focosi and Petrini as "contrary evidences" were, in fact, consistent with or

complementary to our findings. In addition, there are other earlier publications that also demonstrated CD57 as a marker for GC helper T cells (10–12).

Taken together, $CD4^+CD45RO^+CD57^+CXCR5^+$ are currently the best available markers that define Th cells for GC response.

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Comment on "Characterization of Human Lung Tumor-Associated Fibroblasts and Their Ability to Modulate the Activation of Tumor-Associated T Cells"

We read with interest the recent article by Nazareth et al. (1) reporting on the characterization of human lung tumor-associated fibroblasts and their ability to modulate the activation of tumor-associated T cells. We appreciate the newly emerging evidence reporting the impact of the microenvironment on tumor cells in cancer and read with interest the new findings of the above mentioned article on the role of fibroblasts adjacent to tumor cells.

However, we would like to point out that the reported B7-H1 expression by Nazareth et al. (1) in tumor-associated fibroblasts is an in vitro artifact rather than a natural expression phenomenon. We have shown very recently (2) that both normal and tumor-associated fibroblasts obtained from the tissues of breast cancer patients express very low levels (or none) of B7-H1 molecule upon isolation and gradually start to express this molecule upon in vitro culturing of these cells (up-regulation from <5 to 100%). More importantly, fibroblasts ob-

tained from normal people undergoing plastic surgery also demonstrated B7-H1 up-regulation after in vitro culture (up-regulation from 2 to 90%). This phenomenon was found in both fibroblasts and epithelial cells obtained from normal breast as well as foreskin tissues. The B7-H1 up-regulation started from day 1 of culture and peaked on day 10. We have associated the in vitro induction of B7-H1 with cell proliferation because its expression was strongly and significantly associated with the proliferation marker Ki-67. Furthermore, the B7-H1 expression in the fibroblasts was completely diminished after arresting the cells in the G_0 phase (the B7-H1 molecule was up-regulated after in vitro reconditioning of these cells).

The molecular basis for the link between proliferation and B7-H1 expression can be explained by the recent findings of Parsa et al. (3) linking the Akt protein (a kinase important for cellular proliferation) to B7-H1 up-regulation. In addition, our previous in vivo study did not find any significant B7-H1 expression in both normal and tumor-associated fibroblasts analyzed on frozen sections isolated from breast cancer tissues as well as normal breast tissues from plastic surgery patients (4). Similar findings were also reported by Mazanet et al. (5), who demonstrated that B7-H1 was barely detectable in normal untreated skin with only a few keratinocytes and occasional vessels showing very weak staining. Although our study was conducted on breast and skin tissues that are different from the non-small cell lung cancer cells reported in work by Nazareth et al. (1), we believe that up-regulation of B7-H1 upon in vitro culture is a common feature for all types of proliferating cells (2).

Nazareth et al. (1) did not study the expression of B7-H1 in histological sections to show an association of this molecule with the tumor-associated fibroblasts. Study of the expression of B7-H1 molecule in vitro does not reflect the actual situation in the cancer patient. Therefore, we believe that such cultured cells obtained from normal tissue with similar or close passage numbers are the right controls for cultured cells obtained from cancer tissues.

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Response to Comment on "Characterization of Human Lung Tumor-Associated Fibroblasts and Their Ability to Modulate the Activation of Tumor-Associated T Cells"

We have two independently derived sets of new data that establish that B7-H1 is expressed by fibroblasts in the microenvironment of human non-small cell lung tumors. First, our flow cytometry data identify fibroblasts within disrupted lung tumor biopsy tissue through staining with a mAb specific for B7-H1 (clone MIH1). Using an Ab, SM1214P, which is specific for a 112-kDa molecule expressed on human fibroblasts (1–3), we confirmed that the B7-H1^{+} cells were fibroblasts. We found that 42% of the tumor-derived SM1214P^{+} fibroblasts expressed B7-H1 on their cell surface. These B7-H1[+]SM1214P^{+} cells were, as expected, negative for CD45. Second, we demonstrated the presence of B7-H1 on tumor-associated fibroblasts by immunohistochemistry of formalin-fixed lung tumor tissues. Archived human lung tumor sections from our laboratory were stained in the laboratory of Dr. Lieping Chen (Johns Hopkins University, Baltimore, MD) using his anti-B7-H1 mAb clone 5H1, which has been successfully to stain paraffin-embedded formalin-fixed human renal cell carcinomas after Ag retrieval (4). In four of four tumors, distinct membrane staining for B7-H1 was observed in tumor cell subsets (Fig. 1, A and B, asterisks). In two of four tumors, a portion of the cells with a fibroblast morphology, i.e., elongated cells with cytoplasmic extensions and flat/oval nuclei, stained positively with 5H1 (Fig. 1B, arrows). No staining of fibroblasts or tumor cells was observed with an isotype-matched control Ab (Fig. 1C). A trichrome stain of serial sections supports our assumption that these B7-H1^{+} cells were in fact fibroblasts (Fig. 1A). These data confirm our reported results that tumor-associated fibroblasts express B7-H1 on their surface in situ (5) and that this expression is not the result of cell culture as suggested by Drs. Ghebeh and Dermime (6). We recognize that the level of B7-H1 expression in the fibroblasts may vary from one tumor to another and that the level may be influenced by several different factors, including IFN-γ, as we reported (5).

We are pleased that Drs. Ghebeh and Dermime (6) have expressed interest in our work and appreciate their comments, which stimulated us to look more closely for the B7-H1 expression in the tumor-associated fibroblasts before in vitro culture. However, their data that provoked their comments may be open to some questions for two reasons. First, the B7-H1 expression reported in their article was based upon comparatively weak cytoplasmic staining (not membrane staining as has been consistently reported for B7-H1 (4, 7)), and an isotype-matched control Ab was not included (8). Second, these authors failed to use any marker to positively identify their cells as fibroblasts either in vitro or in vivo.

The data presented in our paper extensively characterized primary cultures of tumor-associated fibroblasts phenotypically, and a main conclusion was that this heterogeneous population of cells is able to modulate the response of tumor-associated memory T cells to activation signals to the TCR (5). We establish here with this new data that B7-H1 is expressed on a portion of the tumor-associated fibroblast in situ. Therefore, this subset of cells in the tumor stroma represents a potential regulator of T cell function in the human lung tumor microenvironment.

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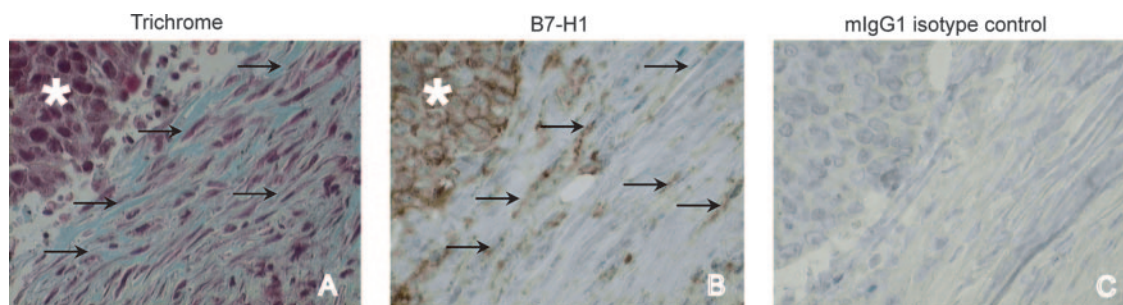
Figure 1. Immunohistochemistry of human non-small cell lung tumor. Serial sections of a representative, formalin-fixed, human non-small cell lung tumor were stained with trichrome (A), B7-H1 (B), or an isotype control Ab (C). A, Trichrome staining of collagen emphasizes the fibroblasts, spindle-shaped cells with elongated nuclei (arrows). B, Anti-B7-H1 identifies a portion of positively-staining fibroblasts (arrows) and tumor cells that stain positively (asterisk). C, Immunohistochemistry with an isotype-control Ab confirms the specificity of the B7-H1 Ab in immunohistochemical staining of formalin-fixed, paraffin sections. Asterisks in each image identify nests of tumor cells in the tumor microenvironment. Magnification, ×400.