CD16$^{low/negative}$ Tumor-Infiltrating Lymphocyte: Lymphoid or Myeloid in Origin?

To the Editors: Although tumor-infiltrating lymphocytes (TIL) are predominantly composed of T lymphocytes, other immune cells also occur within the tumor and may be important in tumor control. We therefore read with interest the study of Schleypen et al. (1) who identified a proportion of TIL in renal cell carcinomas as natural killer (NK) cells. In some tumors, the proportion of such NK-TIL exceeded 20% (high NK-TIL), whereas others had fewer than 20% (low NK-TIL). High NK-TIL were mostly CD16^{positive}. In contrast, low NK-TIL were CD16$^{low\ or\ negative}$. These NK cell populations were not cytolytic to the HLA class I antigen–negative cell line K562; they showed a diversity of CD16 expression and variable ability to become cytotoxic after stimulation. Interestingly, low NK-TIL were cytotoxin^{negative} and perforin$^{dim/negative}$. Our own *in vitro* studies on human NK cell development shed further light on the nature of these NK TIL cells.

We stimulated granulocyte-colony stimulating factor mobilized CD34^{positive} cells from healthy donors with stem cell factor and interleukin-2. After 3 weeks, the cultures generated a heterogeneous CD56^{positive}CD16$^{positive/negative}$ cell population, which phenotypically and functionally resembled the low NK-TIL described in Schleypen et al. article. These cultured cells included two populations: a CD56^{bright}CD16^{negative} and a CD56^{low}CD16$^{low/negative}$ population. They had few or no cytotoxic granules and showed negligible cytotoxicity against K562 and P815 cell lines. Interestingly, the CD56^{low} population expressed the myeloid marker CD33 (2, 3). To further investigate the myeloid origin of CD56^{low}CD33^{positive} cells, these cells were electronically sorted (Fig. 1A) and cultured in the presence of stem cell factor, interleukin-2, interleukin-4, and granulocyte macrophage colony-stimulating factor. After a 10-day culture, these cells underwent a profound morphologic change, rounding up, losing adherence, and acquiring numerous digitations, closely resembling immature dendritic cells (Fig. 1B). Because of technical limitations, we could not analyze their phenotype, but we showed that both lymphoid (CD33^{negative}; ref. 4) and myeloid (CD33^{positive}) populations (2, 3) had a strong antiproliferative effect on the myeloid cell lines K562 and P815.

Given the phenotypic and functional similarities between our *in vitro* cultured cells and low NK-TIL, we propose (*a*) that low NK-TIL may represent the myeloid CD56^{low} population we found in culture and (*b*) that NK-TIL cells can control tumor proliferation through a cytostatic effect, rather than by exerting direct cytotoxicity against the tumor. To determine whether the NK-TIL cells described by Schleypen et al. share identity with the myeloid NK cells we found in culture, further phenotypic and functional analysis (including tests of cytostatic function) on TIL cells would be worthwhile not only in renal cell carcinomas but also in other solid malignancies because infiltrating CD56^{positive} cells have been shown in infiltrating a variety of malignancies to be associated with improved survival rate (5–7).

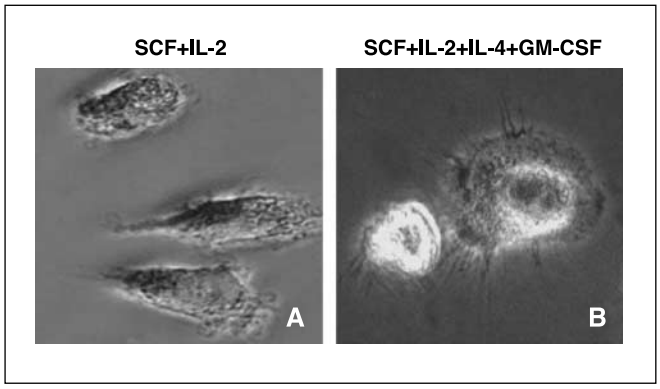

Fig. 1. CD56^{+} myeloid cells acquired dendritic cell characteristics on interleukin-4 and GM-CSF stimulation.

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