To the editor:

Modulation of Th1/Th2 subsets by granulocyte-colony stimulating factor

The recent article published in Blood by Sloord et al.1 suggests that pharmacologic doses of granulocyte-colony stimulating factor (G-CSF) affect cytokine production by lymphocytes in vitro and vivo. Sloord et al. demonstrated that G-CSF decreases interferon (IFN)-γ and increases interleukin-4 (IL-4) production and modulates a balance between T helper 1 (Th1) and T helper 2 (Th2) cells in favor of Th2 cells. Interestingly, a subsequent article by Arpinati et al.2 published in Blood reports that G-CSF mobilizes Th2-inducing dendritic cells (the so-called DC2 cells). Both reports propose that this immunomodulatory effect of G-CSF may be exploited in clinical situations. Sloord et al. suggest the use of this growth factor in conditions associated with Th1/Th2 imbalance such as immune-mediated bone marrow failure syndromes and graft-versus-host disease (GVHD), whereas Arpinati et al. advocate the use of G-CSF-mobilized DC2 cells to induce immune deviation after transplantation of hematopoietic stem cells or organ transplants. As indicated by Liu and Blom.3 in their commentary, the finding of G-CSF-induced mobilization of DC2 cells (and Th2 cells) is a major advancement with several potential clinical applications. However, I would like to point out that none of these reports addresses the relevance and the implications of the above immunomodulatory effect of G-CSF in other clinical situations where this growth factor is widely used.

G-CSF is commonly used to accelerate neutrophil recovery following high dose chemotherapy for various hematogenous and nonhematogenous malignant diseases. The available scientific evidence indicates that the cytokine profile of the T helper cell plays an important role in "cancer immunity."4 The Th1 cells that produce IFN-γ and IL-2 have been shown to exert a powerful antitumor effect, whereas a Th2 profile (IL-4 and IL-10) may have an opposite effect, that is, down-regulation of innate and acquired antitumor immunity. The corollary of these observations is that a Th1 profile is "protective" and therefore beneficial, but a Th2 profile may be deleterious because it may promote tumor growth and dissemination. For this reason, if the findings of Sloord et al. and Arpinati et al. are confirmed, we may have to ask (1) whether the use of G-CSF in malignant conditions is indeed harmful because of the potential risk of tumor growth and dissemination mediated by the Th2 cytokines and, therefore, should be avoided in these situations, and (2) whether an alternative growth factor that predominantly induces a Th1 profile (in addition to the effect on myelopoiesis) such as granulocyte-macrophage colony-stimulating factor (GM-CSF) should be preferred for this purpose. I suppose it may be scientifically logical to suggest that the use of G-CSF should be limited to stem cell mobilization of allogeneic donors (to reduce the risk of graft rejection and acute GVHD), but GM-CSF should be preferred in the treatment of chemotherapy-induced neutropenia in malignant and premalignant diseases (to avoid depression of antitumor activity).

Induction of a Th2 cytokine profile may also be detrimental in certain infections. For example, neutropenia is a relatively common manifestation of acquired immunodeficiency syndrome (AIDS) either as a result of bone marrow damage or due to drug therapy, and G-CSF is used in the management of neutropenic sepsis in these patients. It has been reported that a Th1 to Th2 switch is associated with progression of HIV disease.5 Hence, this growth factor may adversely influence the outcome of this condition by inducing a Th2 cytokine profile.

Should, therefore, the recent findings that G-CSF induces or enhances a Th2 cytokine profile prompt a discussion as to the rational use of colony stimulating growth factors in various clinical situations?

References

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Muttuswamy Sivakumaran
Department of Haematology, Peterborough District Hospital
Peterborough, United Kingdom

To the editor:

Th1/Th2 lymphokine profile of T cells present in the blood of granulocyte-colony stimulating factor–treated stem-cell donors: up or down modulation?

The article by Sloord et al.1 that was published in the April 1 issue of Blood suggests a direct regulatory role of granulocyte-colony stimulating factor (G-CSF) over T cells. The authors show a shift towards Th2, or interleukin 4 (IL-4) production, of CD4+ T lymphocytes from peripheral blood mononuclear cells (PBMCs) obtained from stem-cell donors treated with G-CSF (G-PBMC) on the basis of intracellular detection of IL-4 and interferon (IFN)-γ by flow cytometry. Detection of intracellular lymphokines by flow cytometry requires controls usually not required for detection of surface molecules such as competition of the specific staining with the unlabeled antibody, since isotype control monoclonal antibody (mAb) does not perform well as a negative control for intracellular staining as it does for surface staining.2

To address the same question raised by our colleagues, we...
Figure 1. Cytokine profile of T cells obtained with the same reagents but different fixation time and temperature. (A) PBMCs were fixed with PF for 30 minutes at 4°C (left panels) or 5 minutes at 37°C (right panels) and then intracellular stained. Quadrant settings were based on unstained negative controls. From top to bottom: unstained cells; cells stained with anti–IL-4 FITC, anti–IFN-γ PE (Pharmin-gen, Palo Alto, CA) and anti-CD3 PercP (B&D, San Jose, CA); cells incubated with 25-fold excess of unlabeled anti–IFN-γ and stained as above; cells incubated with 25-fold excess of unlabeled anti–IL-4 and stained as above. The data was collected in a FACSScan and analyzed using CellQuest software (B&D, San Jose, CA). Data gated on the CD3+ population are shown. (B) Comparative analysis of the 2 techniques used on PBMCs obtained before (PBMC) and after (G-PBMC) G-CSF treatment. The data represent mean ± SD obtained from 7 (30 minutes at 4°C) or 5 (5 minutes at 37°C) different stem cell donors for each technique shown.

Zilton F. M. Vasconcelos
Centro Nacional de Transplante de Medula Óssea,
Instituto Nacional do Câncer
Divisão de Medicina Experimental, Coordenação de Pesquisa,
Instituto Nacional do Câncer

Hilda R. Diamond and Daniel G. Tabak
Centro Nacional de Transplante de Medula Óssea,
Instituto Nacional do Câncer

Adriana Bonomo
Divisão de Medicina Experimental, Coordenação de Pesquisa,
Instituto Nacional do Câncer
Departamento de Parasitologia, Universidade de São Paulo

Supported by the Brazilian National Cancer Institute (INCA) and CNPq.

References


Response:

Pharmacologic concentrations of granulocyte-colony stimulating factor affect cytokine expression by lymphocytes

In a manuscript recently published in Blood,1 we demonstrated that lymphocytes stimulated with either CD3 monoclonal antibody (mAb) or phytohemagglutinin (PHA) in the presence of granulocyte-colony stimulating factor (G-CSF) altered the cytokine expression, resulting in a shift in cytokine response from a Th1 to a Th2 pattern. Lymphocytes obtained from healthy individuals following G-CSF mobilization for the purpose of stem cell donation also showed shifted patterns of cytokine expression following coculture with either CD3 mAb or PHA.

We certainly agree with Vasconcelos et al that appropriate...
controls are of paramount importance when performing intracellular staining. In order to ensure that our staining methods were sensitive and specific, we (1) optimized the amount of antibody that was added so as to decrease nonspecific staining, (2) performed neutralizing studies with unlabeled interferon-γ (IFN-γ) and IFN-γ mAb, (3) checked concordance between intracellular staining using antibodies from different manufacturers and by enzyme-linked immunosorbent assay (ELISA) measurements on duplicate samples, (4) measured IFN-γ and interleukin 4 (IL-4) using 2-color staining to ensure that the cell populations did not overlap extensively, and (5) used appropriate isotypic controls.

We found that the addition of excessive anticytokine antibody to the lymphocytes resulted in significant nonspecific staining, particularly when using IL-4 mAb. Results from titration experiments were used to optimize antibody concentrations. In addition, we performed multiple experiments using stimulated and unstimulated lymphocytes from healthy donors: PHA stimulation of cultured cells resulted in a mean of 35% of cells staining with IFN-γ with IFN-γ–fluorescein isothiocyanate (FITC) with a mean channel fluorescence (MCF) of 5.6, whereas isotype controls resulted in an MCF of less than 0.10. Lymphocytes cultured in the presence of IFN-γ or IL-10—stimuli expected to favor development of the Th1 phenotype—stained primarily with IFN-γ, and lymphocytes cocultivated with IL-4 or IL-12—stimuli that favor development of Th2 cells—stained primarily with IL-4. Preincubation of anti–IFN-γ mAb with unlabeled IFN-γ competitively eliminated the staining, as did preincubation of cells with unlabeled IFN-γ mAb; unlabeled irrelevant mAb had no effect. The failure of Vasconcelos et al to observe neutralization with the permeabilization protocol recommended by the manufacturer of the IFN-γ mAb may be related to use of a cold IFN-γ mAb directed at a different epitope than the labeled IFN-γ mAb, or to excess labeled IFN-γ mAb in the sample. To assess the reproducibility of intracellular staining, we also tested another antibody from a different manufacturer (BioSource, Camarillo, CA; Pharmingen, San Diego, CA). Results obtained from comparison of 26 sets of samples showed a highly significant correlation (Pearson correlation coefficient = 0.96). In addition, IL-4 and IFN-γ ELISA measurements of lysed cell samples correlated with the results of intracellular cytokine staining. Measurement of IL-4 and IFN-γ on CD4 cells using 3-color staining showed no overlap between CD4 cells staining with each antibody.

We performed appropriate isotypic controls using labeled antibodies provided by the manufacturer of the cytokine mAbs and as described in Current Protocols in Immunology. Although there are inherent problems in using isotypic controls, they still provide great advantages over unstained cells for negative gating cells. In none of the papers referenced by Vasconcelos et al were unstained cells used as controls, and more recent articles by Gurunathan et al utilize the same isotypic controls as we report in our study.

When we applied the staining method suggested by Vasconcelos et al (a short fixation period at 37°C), we found significant nonspecific uptake of stain in the isotypic control as well as in unstimulated normal cells. When appropriately gated using an isotypic control also prepared in this fashion, there appeared to be no difference in the amount of uptake of stain by the sample when compared to the other fixation method. We believe that the comparison of stained to unstained cells (Figure 1 of the letter by Vasconcelos et al) may therefore not be valid.

With respect to the comments made by Sivakumaran et al, extrapolation of in vitro data for clinical purposes must always be made cautiously. There is no evidence that administration of G-CSF is detrimental in immunosuppressed patients—in fact, quite to the contrary, as multiple trials involving neutropenic HIV-infected patients show that G-CSF consistently decreased the frequency of serious infection. 4, 5 Short courses of G-CSF are especially unlikely to influence tumor progression or chronic infections. Nevertheless, as we describe in the “Discussion” segment of our paper, long-term G-CSF therapy may affect immune processes like graft-versus-host disease or autoimmune marrow destruction.

Elaine Sloand, Sonnie Kim, Jaroslaw Maciejewski, and Neal Young
National Heart, Lung, and Blood Institute
National Institutes of Health, Bethesda, MD

References

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Muttuswamy Sivakumaran