LEUKEMIA LETTER

Tumor Necrosis Factor and Interferon γ Inhibit the Development of Radiation-induced Thymic Lymphomas in C57BL/Ka Mice

Jacques Boniver, Chantal Humblet, and Marie Paule Defresne

Laboratory of Pathological Anatomy and Cytopathology, Department of Pathology, University Hospital of Liege, Liege, Belgium

Recombinant tumor necrosis factor and/or γ-interferon were injected into C57BL/Ka mice after completion of a whole body split dose irradiation, which usually induces thymic lymphomas in more than 90% of the animals. The survival and the incidence of thymic lymphomas were significantly reduced in the cytokine-injected irradiated mice. The protective effect was similar to that obtained by grafting normal bone marrow cells after irradiation. The mechanisms of lymphoma inhibition by TNF or IFN-γ are discussed.

INTRODUCTION

WHOLE body fractionated irradiation (4 × 1.75 Gy at weekly intervals) of C57BL/Ka mice induces thymic lymphomas in more than 95% of the animals (1). The leukemogenic irradiation acts on some subsets of lymphoid cells (2, 3), leading to their transformation into potential neoplastic cells (or "preleukemic cells") which are found in the thymus, and later on in bone marrow, during the latency period preceding the onset of lymphomas (4). For progression to lymphoma growth, preleukemic cells require a thymic microenvironment, since they give rise to tumors only in thymus bearing, but not in thymectomized animals (4, 5).

Interestingly, lymphoma development can be inhibited if a graft of normal bone marrow cells is given early after the split dose irradiation (6). This treatment, however, does not prevent the emergence of preleukemic cells (7, 8), which disappear during the second month (8). The mechanism of lymphoma inhibition by the bone marrow graft is still poorly known. Active thymic repopulation by grafted lymphoid precursors and simultaneous restoration of thymic epithelium have been described and proposed as the protective mechanisms (1, 4, 8, 9).

This marrow-mediated thymic reconstitution might lead to the recovery of intrathymic paracrine secretions, which would result in the elimination of preleukemic cells and, hence, lymphoma prevention. Gamma interferon (IFN-γ) and tumor necrosis factor (TNF) are good candidates for such effects. Indeed, it has been shown that some subsets of thymic cells can produce these cytokines (10, O. Stutman, personal communication).

They also display stimulating effects on several thymic stromal cells including epithelial cells by enhancing class II MHC antigens expression (11, 12, M. P. Defresne et al. in preparation). Furthermore, they are potentially capable of destroying neoplastic cells (13–15); they can even act in synergy (15).

As a preliminary approach in studying the possible role of IFN-γ and TNF-α as mediators of the protective role of bone marrow grafting, we determined the incidence of thymic lymphomas in mice that had been inoculated with IFN-γ or TNF-α after a leukemogenic split dose irradiation. We observed a significant reduction of mortality and of thymic lymphoma incidence.

MATERIALS AND METHODS

Animals. C57BL/Ka mice originating from Stanford University (Stanford, CA) were raised in our animal colony.

Irradiations. Five- to 6-week-old female mice were whole body irradiated with 4 doses of 1.75 Gy applied at weekly intervals. The conditions were: Stabilivolt Siemens, 190 kv, 18 mA, 0.5 mm Cu filter, focal distance of 35 cm, dose rate: 1.6 Gy/min.

IFN-γ and TNF-α Treatments. Murine r-IFN-γ (2.10^7 U/mg) and human r-TNF-α (6.10^7 U/mg), produced by Genentech and kindly provided by Boehringer Ingelheim International, were diluted in RPMI 1640 supplemented with 10% fetal calf serum; 200-μl aliquots containing either 4×10^4 U IFN-γ or 2.5×10^5 U TNF-α or a mixture of both were injected i.p. on day 1 after the last 1.75 Gy irradiation. Three injections per week (one every second day) during the first 6 weeks were performed. There were 16 mice in each experimental group. A group of 16 irradiated mice was used as control.

Statistical Analysis. The survival curve was established according to Kaplan and Meier (16). The Mantel-Haentzel test (17) was used to compare the differences between the various experimental groups.

RESULTS

The curves in Figures 1 and 2 show the survival and incidence of lymphomas in the TNF-α and IFN-γ inoculated irradiated mice as compared with control irradiated animals. In this representative observation, there were 16 mice in each experimental group.

The incidence of thymic lymphomas was significantly lower in the three groups of mice that received cytokine injections than in the only irradiated animals ($p < 0.05$). There was no significant difference between the three groups of cytokine-injected mice. Similar observations were made for survival.

DISCUSSION

We report here that repeated injections of r-TNF-α or r-IFN-γ after a leukemogenic split dose irradiation prevents the onset of thymic lymphomas in C57BL/Ka mice. These effects are reminiscent of those obtained by a bone marrow transplantation (6) or, to minor extent, after inoculation of sheep spleen extracts (18) or supernatants of Newcastle disease virus infected fibroblasts (with a β-interferon type antiviral activity) (19).

Received March 20, 1989. Accepted April 25, 1989.

Correspondence to: Prof. J. Boniver, Laboratory of Pathological Anatomy and Cytopathology, Department of Pathology, University Hospital of Liege, B 4000, Liege, Belgium.

0887-6924/89/0308-0611$2.00/0
LEUKEMIA

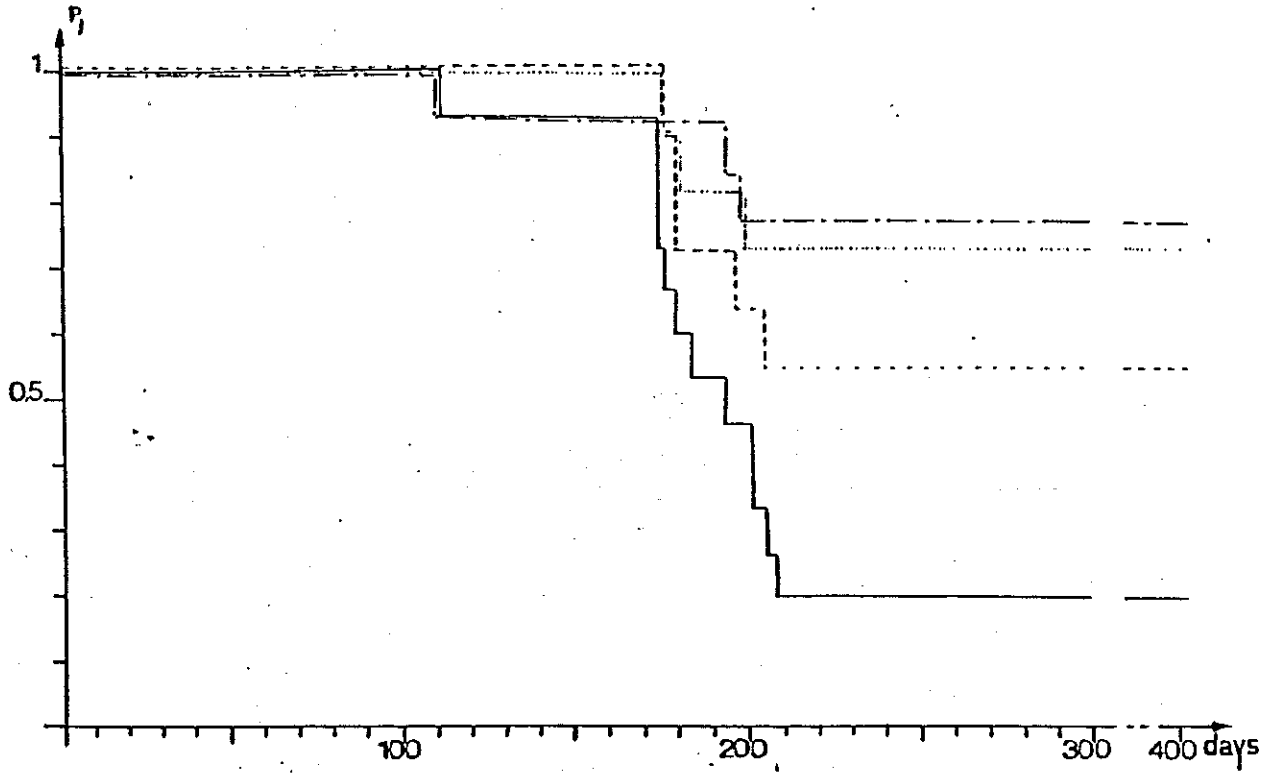

Figure 1. Survival of 4 × 1.75 Gy irradiated C57BL/Ka mice: controls (—); + IFN-γ (– – –); + TNF-α (···) + IFN-γ and TNF-α (·····). P_j represents the probability of survival, according to Kaplan and Meier (17).

The present observations clearly demonstrate that IFN-γ and TNF-α, besides their well-documented anticancerous effects in vitro and in vivo (12–14), can also act on *preneoplastic* tissues and thus disrupt the multistep process, which, in this experimental system, leads to the development of lymphomas. Whether the cytokines, which are produced by cells of the immune system, act on the preleukemic cells indirectly or directly remains to be solved.

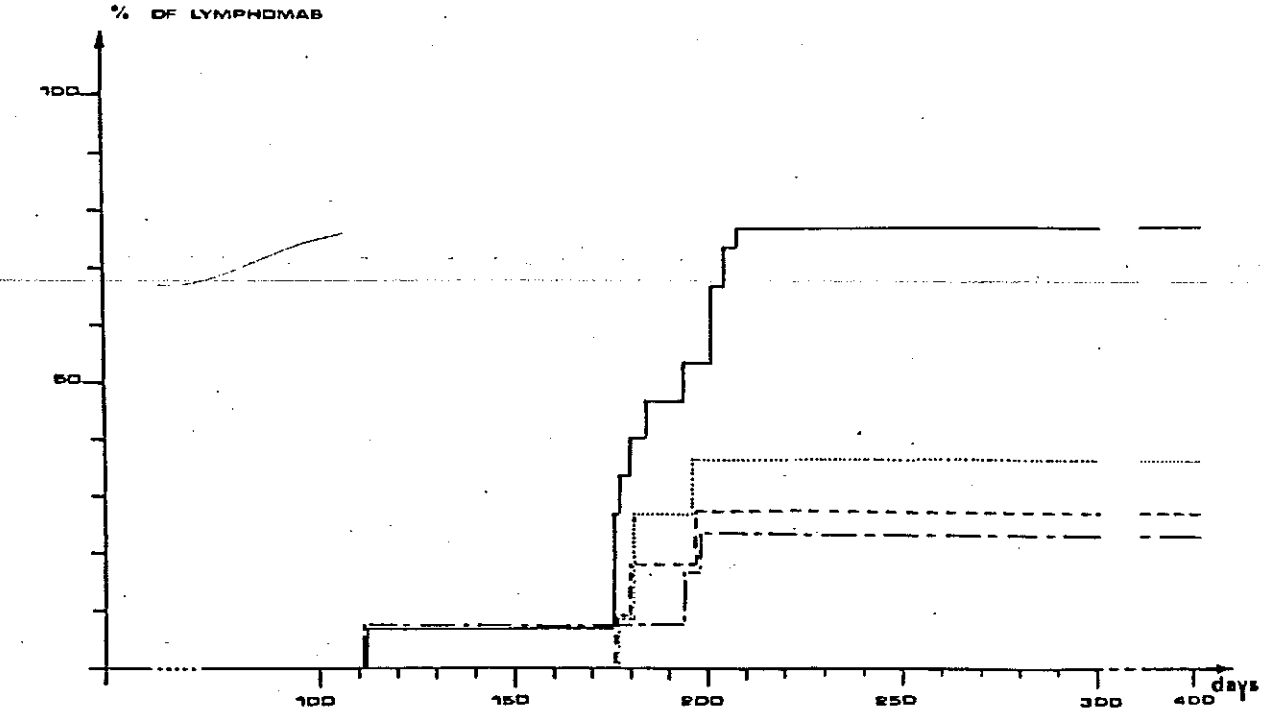

Figure 2. Cumulative lymphoma incidence in 4 × 1.75 Gy irradiated C57BL/Ka mice: controls (—); + IFN-γ (– – –); + TNF-α (···); + IFN-γ and TNF-α (·····).

In favor of a direct mechanism is the fact that several types of neoplastic cells are susceptible to the cytotoxic effects of TNF-α (12, 13). A similar effect on preneoplastic cells has not yet been described. Alternatively, the protective effect might be indirect.

We have recently shown that both IFN-γ and TNF-α increase the frequency of interactions between immature thymocytes and thymic epithelial cells, most likely through MHC antigens overexpression (20).

As lymphoepithelial interactions are altered in the preleukemic thymuses of irradiated mice, one can postulate that TNF-α and IFN-γ restore them; preliminary data argue in favor of this view (M. P. Defresne et al., in preparation). If further experiments confirm these observations, it still has to be explained how the restoration of lymphoepithelial interactions results in the elimination of preleukemic cells.

One should also ask whether the well-known protective effect of bone marrow grafting against radiation-induced lymphomas is related to IFN-γ and TNF-α. The leukemogenic split dose irradiation might deplete the IFN-γ or TNF-α producing thymic cells, since a depletion of CD4+ cells (21) and macrophages (N. Collignon et al., in preparation) has been observed. The bone marrow graft, which provides precursors of all hematopoietic cells, including T cells and macrophages, might restore these thymic populations and hence the intrathymic production of both cytokines. The fact that bone marrow transplantation acts on lymphoepithelial interactions (8) in the same way as TNF-α or IFN-γ pleads in favor of these hypotheses, which are now submitted to investigation in our laboratory.

We propose that the cytokine-mediated inhibition of the multistep process of carcinogenesis in this model is of interest for developing new strategies leading to the eradication of preneoplastic lesions in human patients and in preventing recurrences after treatment of cancers.

Acknowledgments. We thank Miss M. Basiglini for preparing the manuscript. This work was supported by the Belgian Fund for Medical Scientific Research, the Centre Anticancéreux près l'Université de Liège, and Boehringer Ingelheim International. M. P. D. is a Research Associate of the National Fund of Scientific Research.

REFERENCES

1. Kaplan HS. On the natural history of the murine leukemias. Presidential address. Cancer Res 1967;27:1325–1340.
2. Kaplan HS. The role of cell differentiation as determinant of susceptibility to virus carcinogenesis. Cancer Res 1961;21:981–983.
3. Boniver J, Simar LS, Courtoy R, Betz EH. Quantitative analysis of thymus lymphoid cells during murine radioleukemogenesis. Cancer Res 1978;38:52–58.
4. Boniver J, Decleve A, Lieberman M, Honsik C, Travis M, Kaplan HS. Marrow thymus interaction during radiation leukemogenesis in C57BL/Ka mice. Cancer Res 1981;41:390–392.
5. Haran-Ghera N. Dependent and autonomous phases during leukemogenesis. Leukemia Res 1978;2:11–21.
6. Kaplan HS, Brown MB, Paull J. Influence of bone marrow injection on involution and neoplasia of mouse thymus after systemic irradiation. JNCI 1953;14:303–316.
7. Haran-Ghera N. Pathways in murine radiation leukemia-genesis-coleukemogenesis. In: Yuhas J, Tennant RW, Regan JD, eds. Biology of radiation: carcinogenesis. New York: Raven Press, 1976:245–260.
8. Defresne MP, Greimers R, Lenaerts P, Boniver J. Effects of bone marrow grafting on preleukemic cells and thymic nurse cells in C57BL/Ka mice after a leukemogenic split dose irradiation. JNCI 1986;77:1079–1085.
9. Van Bekkum DW, Boersma WJA, Eliason JF, Knaan S. The role of prothymocytes in radiation-induced leukemogenesis in C57BL/Rij mice. Leukemia Res 1984;8:461–471.
10. Papiernik M, Dombret H, Stefanos S, Wietzerbin J. Control of Ia antigen expression on phagocytic cells of the thymic reticulum by Interferon-gamma and prostaglandins. Eur J Immunol 1986;16:296–300.
11. Lo D, Sprent J. Exogenous control of I.A expression in fetal thymus explants. J Immunol 1986;137:1772–1775.
12. Berry SF, Arenzana-Seisdebos F, Cohen S, Devos R, Charron D, Virelizier JL. Interferon modulates HLA class II antigen expression on cultured human thymic epithelial cells. J Immunol 1985;135:1165–1171.
13. Ruddle NH. Tumor necrosis factor and related cytokins. Immunol Today 1987;8:129–130.
14. Beutler B, Cerami A. Cachectin: more than a tumor necrosis factor. N Engl J Med 1987;316:379–382.
15. Tsujimoto M, Yip YK, Vilcek J. Interferon-γ enhances expression of cellular receptors for tumor necrosis factor. J Immunol 1986;136:2441–2444.
16. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Statis Assoc 1958;53:457–481.
17. Peto R, Pike MC, Armitage P, Brescon NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PC. Design and analysis of randomized clinical trials requiring prolonged observations of each patient. Analysis and examples. Br J Cancer 1977;35:1–39.
18. Berenblum T, Cividalli G, Trainin N, Hodes ME. Some properties of "RLP": a factor from sheep spleen capable of inhibiting radiation leukemogenesis in mice. Blood 1965;26:8–19.
19. Lieberman M, Merigan TC, Kaplan HS. Inhibition of radiogenic lymphoma development in mice by interferon. Proc Soc Exp Biol Med 1971;138:575–578.
20. Defresne MP, Humblet C, Rongy AM, Boniver J. Control of lymphoepithelial interactions within thymic nurse cells by interferon-gamma and tumor necrosis factor. Possible role in the modulation of intrathymic education? Thymus, 1989 (in press).
21. Rongy AM, Humblet C, Lelievre P, Greimers R, Boniver J. Abnormal thymocyte subpopulations in split dose irradiated C 57 BL/Ka mice before the onset of lymphomas. Effects of bone marrow grafting. Thymus; submitted.