TNF-α and Radiation-Induced Thymic Lymphomas

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INTRODUCTION

In C57BL/Ka mice, a split-dose regimen of whole body x-ray irradiations (4 X 1.75 Gy at weekly intervals, starting at one month of age) induces a high incidence of thymic lymphoblastic lymphomas (>90%) (1). During the 3-9 month latency period preceding the onset of lymphoma, preleukemia cells (PLCs) are detected in the thymus, and also in bone marrow. As shown by the *in vivo* transplantation assay used for their detection (2,3), these preleukemia cells depend upon thymic microenvironment for their progression towards lymphoma transformation (2, 4). PLCs most likely belong to the immature subsets of thymocytes (5-8), but the factors promoting their neoplastic conversion are still poorly known. The deep alterations to the prethymic and intrathymic cell populations observed after the split-dose irradiation regimen may contribute to this promotion through a disturbance to the regulation of thymocyte proliferation and differentiation; bone marrow prothymocyte activities are reduced (4,8-10) intrathymic thymocyte precursors with self renewal capacities and abnormal differentiation emerge (7), and the thymic microenvironment, particularly some epithelial cells, the thymic nurse cells (TNCs) (11), and dendritic cells (12) are altered. An approach to solve this question has been to study the mice which are given a normal syngeneic bone marrow graft after irradiation. Such a graft inhibits the onset of thymic lymphomas (13). However, PLCs are transiently present in the thymus (11), which is actively repopulated by grafted marrow derived cells, whereas the abnormal lymphoid cell populations disappear and the epithelial and dendritic cells are restored (7, 8, 9, 11). Thus, one may expect that this thymus restoration, or at least one of its components, contributes to the elimination of PLCs and to lymphoma prevention. We have proposed that bone marrow grafting might induce the production of cytokines, such as interferon-γ (IFN-γ) or tumor necrosis factor-α (TNF-α), or others, which would then eliminate PLCs directly or indirectly (14).

The present paper reports some selected data which indicate that exogenous TNF-α or IFN-γ, injected into split-dose irradiated mice, mimic several effects of bone marrow grafting, i.e., inhibition of lymphoma, elimination of PLCs, thymocyte and TNC restoration. Furthermore, we present some preliminary studies which indicate that bone marrow grafting induces TNF-α production in the thymus and that its effect can be partially inhibited by injection of anti-TNF-α antibodies.

RESULTS AN DISCUSSION

Exogenous TNF-α or IFN-γ mimic the effects of bone marrow grafting in split-dose irradiated mice (Table I): As shown in the table and as previously published (12, 15), the *incidence of thymic lymphomas* in C57BL/Ka mice which were given a normal bone marrow graft or exogenous TNF-α, was strikingly reduced; IFN-γ was slightly less efficient.

This inhibition of lymphoma development is related to the disappearance of *preleukemia* cells from the thymus;, whereas more than 90% of irradiated mice contained thymic PLCs on day 60 after irradiation, such cells were detected in less than 10% of bone marrow grafted or cytokine injected animals.

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Simultaneously, *thymocyte differentiation* was restored. In the 4 x 1.75 Gy-treated animals, there was a significant increase of the CD4-CD8- thymocytes, whereas the proportion of CD4+CD8+ cells dropped (7). Conversely, in TNF-α injected animals, as well as in bone marrow grafted mice, the percentages were first also altered but later on they returned to normal values.

Some components of the thymic microenvironment were also partially restored, as assessed by the estimation of the numbers of *thymic nurse cells*. In fact, after the last irradiation, the numbers of TNCs were very low in all the experimental groups of irradiated mice (< 5,000/thymus versus > 15,000/thymus in normal mice) (11). Later on, from day 45 onwards, TNC numbers increased in cytokine-injected animals to reach values intermediate between those seen in normal mice and in irradiated animals. In bone marrow grafted animals, the restoration was complete.

The observations on cytokine-inoculated mice raise many questions, such as the mechanisms leading to the partial restoration of thymic nurse cells and thymocyte differentiation. Hence, the effects on thymocyte differentiation observed might be due to the restoration of the precursor compartment in bone marrow; this mechanism is now under investigation. The restoration of TNCs might be a consequence of thymocyte restoration, since TNCs result from interaction between immature thymocytes and epithelial cells (11). Furthermore, a direct effect on the epithelial cells might be involved since TNF-α and IFN-γ can enhance *in vitro* the interactions between thymocyte precursors and epithelial cells leading to the formation of TNC (15).

Evidence for TNF-α production and activity in bone marrow grafted irradiated mice: By using *in situ hybridization*, TNF-α mRNAs can be detected in a few thymic cells. The numbers of TNF-α-*producing cells in the thymus* vary during fetal life, suggesting a role for this cytokine in thymocyte ontogeny (17). In adult mice as well as in split-dose irradiated animals, the number of TNF-α-mRNA-producing cells is very low (about 1 positive cell per mm^2 of thymus section). In bone marrow grafted animals, there was an increase of positive cells, which was highly significant on day 30 after the last irradiation (>8 cells/mm^2) (17).

The biological effects of endogenous TNF-α for lymphoma prevention in bone marrow grafted mice is supported by the effects of injected rabbit anti-TNF-α antibodies (0.5 mg/week for 6 weeks) on lymphoma incidence in marrow grafted animals. The preliminary data presently available indicate that, whereas the incidence of thymic lymphomas was about 2-5% in marrow grafted animals, it was increased to 40% in the marrow grafted animals which have been treated with anti-TNF-α antibodies.

Endogenous TNF-α might thus be involved in the biological effects after bone marrow grafting. The level of TNF-α mRNA producing cells observed in the thymus of marrow grafted animals was quite similar to that observed transiently in the fetal thymus (16). TNF-α is thought to contribute to the regulation of thymocyte expansion, in synergy with other cytokines (18). It might also be involved in selection mechanisms, involving apoptosis, at some stages of ontogeny (16). In marrow grafted animals, similar mechanisms might be mediated by TNF-α and contribute to lymphoma prevention. This view is supported by the preliminary data obtained with rabbit anti-TNF-α antibodies.

Altogether, our data, which have to be completed, support the proposed hypothesis that bone marrow grafting inhibits the onset of radiation -- induced thymic lymphomas by acting on the cytokine network in the thymus. Whether these cytokines act on preleukemic cells directly or indirecty has still to be defined. Furthermore, whether alterations to

Table I. Comparison of Effects of TNF-a and IFN-g Treatments and Bone Marrow Grafting in Split-Dose Irradiated Mice

Mouse treatment		Incidence of lymphomas[d]	Preleukemic cells[e] Incidence of lymphomas in recipient animals	Thymocyte differentiation[f]				Thymic[h] nurse cells
4X1.75 Gy[a]	Further treatment			CD4-CD8-	CD4+CD8+	CD4+CD8-	CD4-CD8+	
-	-	0	0	3.1%	83%	8.9%	5%	17000
+	-	90%	90%	14.5%	46%	28.8%	10.7%	3000
+	BM graft[b]	5%	0	4.5[g]	84[g]	8.5[g]	3[g]	19000
+	mTNFα[c]	12%	5%	3.2%	82%	9.1%	5.7%	9000
+	mIFNγ[c]	30%	4%	3.3%	83.6%	8.6%	4.5%	12000

[a]One-month old C57BL/KA mice were given four whole-body X-irradiations of 1.75 Gy (11). [b]10^7 bone marrow cells collected from normal congenic Thy1.1 C57BL/Ka mice were intravenously injected within 2 h after the last 1.75-Gy irradiation (11). [c]mTNF-α, three intraperitoneal inoculations of 25,000 U/week, for 6 weeks, starting after the last irradiation (13); mIFN-γ, 40,000 U/injection, the same number of injections as for mTFN-α (13). [d]Established on day 300 after last irradiation. [e]Preleukemia-cell activity of thymuses removed after the last irradiation, estimated by the frequency of thymic lymphomas of donor type observed in recipient animals (11). [f]Percentage of thymocytes expressing CD4 and/or CD8 on day 60 after irradiation (7). [g]From Rongy et al. (7). [h]Number of thymic nurse cells/thymus on day 60 after irradiation (11).

such networks in split-dose irradiated mice may be the promoting factors for preleukemia cells is now under investigation.

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REFERENCES

1. Kaplan HS. On the natural history of the murine leukemias. Cancer Res 1967 ; 27 : 1325-1340.
2. Haran Ghera N, Rubio N, Leef F, Goldstein G. Characteristics of preleukemia cells induced in mice. Cell Immunol 1978;37:308-314.
3. Goffinet G, Defresne MP, Boniver J. Correlation of alkaline phosphatase activity to normal T cell differentiation and to radiation leukemia virus-induced preleukemic cells in the C57 BL mouse thymus. Cancer Res 1983;43:5416-5426.
4. Boniver J, Decleve A, Lieberman M, Honsik C, Travis M, Kaplan HS. Marrow thymus interactions during radiation leukemogenesis in C57 BL/KA mice. Cancer Res 1981;41:390-392.
5. Majumdar AS, Guidos C, Kaneshima H, White JH, Mariam J, Lieberman M, Weissman IL. An immunodominant murine lymphoma cell surface heterodimer marks thymic progenitor subsets. J Immunol 1990;144:111-121.
6. Hays EF, Bristol GC, Lugo JP, Wang XF. Progression to development of lymphoma in the thymus of AKR mice treated neonatally with SL 3-3 virus. Exp Hemat 1989;1117:1116.
7. Rongy AM, Humblet C, Lelievre P, Greimers R, Defresne MP, Boniver J. Abnormal thymocyte subpopulations in split dose irradiation C57 BL/Ka mice before the onset of lymphomas. Effects of bone marrow grafting. Thymus 1990;16:7-27.
8. Pazmino NH, Mc Evan R, Ihle JN. Radiation leukemia in C47 BL/6 mice III. Correlation of altered expression of terminal deoxynucleotidyltransferase to induction of leukemia. J Exp Med 1978;148:1338-1350.
9. Van Bekkum DW, Boersma WJA, Eliason JF, Knaan S. The role of prothymocytes in radiation-induced leukemogenesis in C57 BL/Rij mice. Leukemia Res 1984;8:461-471.
9. Van Bekkum DW, Boersma WJA, Eliason JF, Knaan S. The role of prothymocytes in radiation-induced leukemogenesis in C57 BL/Rij mice. Leukemia Res 1984;8:461-471.
10. Muto M, Kubo E, Sado T. Cellular events during radiation-induced thymic leukemogenesis in mice : abnormal T cell differentiation in the thymus and defect of thymocyte precursors in the bone marrow after split-dose irradiation. J Immunol 1985; 134:2026-2031.
11. Defresne MP, Greimers R, Lenaerts P, Boniver J. Effects of marrow grafting on preuleukemic cells and thymic nurse cells in 57BL/Ka mice after a leukemogenic split dose irradiation. J Natl Cancer Inst 1986 ;77:1079-1085.
12. Sprecher E, Giloh H, Rahamim E, Yefenof E, Becker Y. Cytofluorometric analysis of thymic interdigitaling cells from C57BL16 mice prior to and after leukemogenic x-irradiation. Leuk Res 1989;13:799-809.
13. Kaplan HS, Brown MB, Paull J. Influence of bone marrow injection on involution and neoplasia of mouse thymus after systemic irradiation. J Natl Cancer Inst 1953;14:303-316.
14. Boniver J, Humblet Ch, Defresne MP. Tumor necrosis factor and interferon γ inhibit the development of radiation-induced thymic lymphomas in C57BL/Ka mice. Leukemia 1989; 3 : 611-613.
15. Defresne MP, Humblet C, Rongy AM, Greimers R, Boniver J. Effect of interferon-gamma and tumor necrosis factor-alpha on lymphoepithelial interactions within thymic nurse cells. Eur J Immunol 1990;20:429-432.
16. Deman J, Martin MT, Delvenne P, Humblet C, Boniver J, Defresne MP. Analysis by in situ hybridization of cells expressing mRNA for tumor necrosis factor in the developing thymus of mice. Developmental Imm, in press.
17. Delvenne Ph, Defresne MP, Humblet C, Martin MT, Rongy AM, Courtoy R, Greimers R, Delvenne C, Paulus JM, Grosdent JC, Boniver J. *In situ* hybridization studies on the TNF-α in the mouse thymus : role of tumor necrosing factor α in the prevention of radiation-induced thymic lymphomas. In: Imhof BE, Berrih-Aknin S, Ezine S, eds. Lymphatic tissues and *in vivo* immune responses. New York, Basel, Hong-Kong Marcel Dekker, 1991:33-35.
18. Suta T, Murray R, Guidos C, Zlotinik A. Growth-promoting activity of Il-1α, Il-6 and tumor necrosis factor-α in combination with Il-2, Il-4 or Il-7 on murine thymocytes: differential effects on CD4/CD8 subsets and on CD3+/CD3- double-negative thymocytes. J Immunol 1990;144:3039-3045.