readily available and can more rapidly reconstitute lympho-hematopoiesis than bone marrow stem cells (*17*).

These observations suggest a role for bFGF in hematopoiesis. This growth factor, produced by a variety of cell types (fibroblasts, endothelial cells, and macrophages), is mitogenic to many cells, including fibroblasts and capillary endothelial cells (*18*). In *Xenopus* blastula it can induce cells from the animal pole to develop as mesoderm (*19*) and as ventral derivatives, including blood cells. Our studies indicate a synergistic interaction between bFGF and multilineage CSFs (IL-3, GM-CSF) on early adult hematopoietic progenitors; both CSFs and bFGF have been postulated to act on target cells after binding to the extracellular matrix, particularly heparan sulfate (*20*). We suggest that bFGF may have a role in the control of proliferation or differentiation, or both, of early adult hematopoietic progenitors, as it apparently does in embryonic hematopoietic development in *Xenopus* (*19*).

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10. We evaluated expression of cell surface markers by indirect immunofluorescent flow cytometry. Cells (1×10^5) were incubated with MAbs Leu4, Leu12, Leu19, LeuM3, T9 (anti-transferrin receptor) BI3C5, HPCA-I, or antibody to HLA-DR (Becton-Dickinson). After three washes and incubation with fluorescein isothiocyanate-conjugated $F(ab')_2$ fragments of goat antibody to mouse immunoglobulin, cells were washed, resuspended in Hank's formalin solution, and analyzed by FACScan (Becton-Dickinson).
11. The clonogenic assays were performed as follows. Step I progenitors. In FCS^+ cultures, PBMCs (step IA) were cultured (3×10^5 cells/ml per dish at least two plates per point) in 0.9% methylcellulose, 40% FCS (Flow), and Ep (3.0 IU/ml) in Iscove's modified Dulbecco's medium supplemented with γ-thioglycerol (10^{-4} M) (Sigma) at 37°C in a humidified atmosphere of 5% CO_2/5% O_2/90% air. Colony scoring was performed on day 14 of culture (*6*). For FCS^- cultures, FCS was replaced by a mixture of (i) >98 to 99% purified BSA, (ii) human low-density lipoprotein, (iii) pure human transferrin, and (iv) a mixture of pancreatic bovine insulin, nucleosides, rare inorganic elements, sodium pyruvate, and L-glutamine (*6*). The dishes (at least two plates per point) were incubated as for FCS^+ cultures. Colonies were scored 1 to 2 days later than FCS^+ culture (*6*). In step II to step IV progenitors, the FCS^+ and FCS^- culture conditions were as described above, with the following exceptions. (i) Progenitors were seeded at a concentration of 1×10^5 (step II) or 1×10^2 (step III and step IV) cells/ml per dish. In step III and step IV assays, control unicellular cultures provided equivalent results. (ii) Several CSFs were added at optimal concentrations to sustain the formation of CFU-GEMM, BFU-E, and CFU-GM colonies, that is, IL-3 (100 U/ml), GM-CSF (10 ng/ml), and Ep (3.0 U/ml). Control experiments were performed with addition of IL-3 and GM-CSF only. In experiments presented in Fig. 2, A and B, bFGF was also added at the indicated concentration.
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15. The dose-response curves were performed in FCS^- clonogenic culture seeded with step IV cells treated with recombinant aFGF (R&D Systems) (0.1 to 200 ng/ml), recombinant platelet-derived growth factor (Amgen) (0.1 to 10 ng), recombinant epidermal growth factor (Peninsula Laboratories) (1 to 100 ng), recombinant insulin-like GF-1 (Amgen) (1 to 20 ng), or transforming GF-β2 (>95% purified, R&D Systems) (0.01 to 10 ng).
16. In a typical experiment on the buffy coat from a single donor, the cell number and progenitor yield were 8×10^8 cells and 100% yield (step IA), 0.48×10^8 and 79% (step II), 8×10^5 and 77% (step III), and 1.0×10^5 and 33% (step IV).
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22. We thank S. Peddis for technical assistance, F. Cionni and M. E. Ganci for secretarial assistance, and D. Marinelli for editorial help and graphics. Supported in part by Progetto Italia-USA Terapia dei Tumori, Istituto Superiore di Sanità, Rome, and Progetto finalizzato Biotechnologie (grant no. 89.00256.70 to C.P.), CNR, Rome, Italy. Recombinant human IL-3 (specific activity, 4×10^6 U/mg) and GM-CSF (1.7×10^7 U/mg) were supplied by S. Clark (Genetics Institute, Pilot Development Laboratory). Recombinant human Ep (1.1×10^5 U/mg) was provided by Amgen.

12 February 1990; accepted 5 July 1990

Protection from Chemotherapy-Induced Alopecia in a Rat Model

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Alopecia (hair loss) is among the most distressing side effects of cancer chemotherapy. Little progress has been made, however, in its prevention or treatment, partly because of the lack of suitable experimental model. In recent work on the treatment of myelogenous leukemia in the rat, the following observations were made: (i) treatment of 8-day-old rats with cytosine arabinoside consistently produced alopecia, and (ii) ImuVert, a biologic response modifier derived from the bacterium *Serratia marcescens*, uniformly produced complete protection against the alopecia. In subsequent experiments, both cyclophosphamide and doxorubicin also produced alopecia in this model, and the doxorubicin-induced alopecia was prevented by treatment with ImuVert. The potential relevance of these observations to chemotherapy-induced alopecia in the clinical setting should be examined.

ALOPECIA IS A FREQUENT AND DISTRESSING side effect of many clinically active chemotherapeutic agents, often causing patients to refuse potentially curative chemotherapy (*1–5*). Although this complication has been known for many decades (*3*), little progress has been made in its prevention or treatment (*6–9*), in part because of a lack of a suitable, reproducible, experimental model. Although doxorubicin-induced hair loss has been described in the Angora rabbit (*10*), its severity is assessed by weighing the amount of hair that regrows after shaving or the amount of hair shed upon grooming. Recently we have been studying the efficacy of chemotherapy in

combination with the biologic response modifier ImuVert in the treatment of leukemia (*11*), using transplanted rat myelogenous leukemia (*12, 13*) as model. ImuVert, a biologic response modifier, is a membrane vescicle-ribosome preparation derived from the bacterium *Serratia marcescens* by a series of lytic and centrifugal steps, including sedimentation on a sucrose gradient (*14*). The composition and activity of the final product, measured as stimulated natural killer (NK) cell activity relative to α-interferon, are highly reproducible with only slight variability. In this model, successful transplantation of leukemia requires the use of young (6- to 8-day-old) rats. We observed that these young rats developed severe generalized alopecia regularly in response to cytosine arabinoside (ARA-C), but animals receiving ARA-C in combination with ImuVert were virtually completely protected from alopecia.

Six hours after intraperitoneal injection of chloroleukemia cells (*15*), animals were treated with ARA-C in the presence or absence of ImuVert (25 μg). This ImuVert dose was chosen on the basis of in vitro monocyte stimulation data (*11*) as well as toxicity studies in young rats. All animals had a near full coat of hair at the start of the experiment. On day 6 of treatment, all rats in group II (ARA-C alone) had alopecia with hair loss starting over the head and rapidly progressing to include the entire body. Sixteen of the 23 rats had total hair loss, another 4 animals had lost over 50% of their hair, and 3 animals had mild alopecia (Table 1). In sharp contrast, alopecia was totally absent in 15 of the 20 rats in group IV (Fig. 1) and was mild in the remaining 5. Rats in group III were indistinguishable from controls. Examination of skin biopsies showed profound loss of hair follicles in the ARA-C–treated rats but no loss of follicles in ARA-C + ImuVert group (Fig. 2). The incidence of death from chloroleukemia in the four groups is also shown in Table 1. All rats in group I were dead of chloroleukemia by day 18. Two of 23 rats (9%) in group II, 9 of 20 (45%) in group III, and 18 of 20 (90%) in group IV have remained disease-free.

In two separate experiments, similar results were obtained in 8-day-old rats not bearing chloroleukemia (Table 2). Protection from ARA-C–induced alopecia by ImuVert was observed at ImuVert dosages of both 25 and 10 μg.

These observations with ARA-C prompted examination of two other chemotherapeutic agents known to produce alopecia in humans: doxorubicin (DX) and cyclophosphamide (CTX). Twenty 8-day-old rats were treated with DX [2 mg per kilogram of body weight per day intraperitoneally (IP)] without or with ImuVert [25 μg subcutaneously (SC)] for 7 days. All rats receiving DX alone had complete alopecia over the head and proximal part of the neck. None of the animals given ImuVert had alopecia (Table 3, experiment 1) (Fig. 3). In another experiment, animals were treated with a single dose of CTX (25 mg/kg IP) without or with ImuVert (25 μg/day IP for 7 days). Four of ten rats treated with CTX alone had total body alopecia indistinguishable from that produced by ARA-C (not illustrated); four others had moderately severe alopecia (loss of more than 50% of body hair); and the remaining two rats had mild alopecia. A similar pattern was noted in the animals treated with CTX + ImuVert; no protection against alopecia was observed with ImuVert (Table 3, experiment 2).

These data suggest that the young rat may serve as an excellent animal model for chemotherapy-induced alopecia. All three agents examined in this study and known to produce alopecia in the clinical setting, ARA-C, CTX, and DX, produced alopecia in the young rat. Both ARA-C and CTX produced generalized body alopecia, whereas alopecia from DX was limited to the head and proximal neck. In our study, the age of the rat appeared to be critical; repeated attempts to induce alopecia with ARA-C in adult rats were unsuccessful. Compared to the Angora rabbit, the young rat offers the advantages of a high degree of reproducibility with grossly visible and often total hair loss, greater availability, and convenience for large-scale testing.

ImuVert offered virtually complete protection from alopecia induced by ARA-C and DX. However, because of DX toxicity in young rats, several dose schedules had to be tested, and, even at 2 mg/kg, most of the

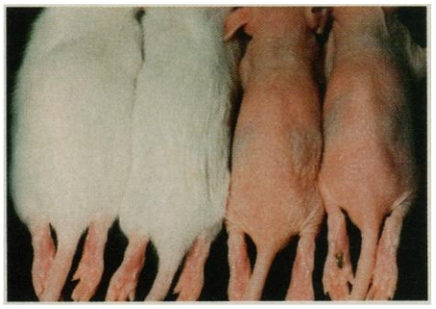

Fig. 1. Color photographs of two rats each from the ARA-C–only group (group II) (right) and from the ARA-C + ImuVert group (group IV) (left) taken on day 15 of the experiment. Treatment schedule was as described in the legend to Table 1. ImuVert provided complete protection against ARA-C–induced alopecia.

Table 2. ARA-C–induced alopecia in normal rats. In experiment 1 38 8-day-old rats were randomly divided into two groups of 19 rats each. Group I received ARA-C (25 mg/kg per day IP) for 7 days, and group II received ARA-C in the same dose plus ImuVert (25 μg/day SC) for 7 days. Data were recorded on day 9 of the experiment (2 days after ARA-C treatment was stopped). Experiment 2 was carried out as in experiment 1, except that there were 12 rats per group and the ImuVert dose was 10 μg/day. Protection from ARA-C–induced alopecia was observed both at the 25- and at the 10-μg dose of ImuVert.

Group	Alopecia			
	0	1+	2+	3+
Experiment 1				
I (ARA-C)	0	0	8	11
II [ARA-C + ImuVert (25 μg)]	14	5	0	0
Experiment 2				
I (ARA-C)	0	0	5	7
II [ARA-C + ImuVert (10 μg)]	10	2	0	0

Table 1. Occurrence and severity of alopecia in groups I to IV. Eighty-four 8-day-old Fischer rats were injected with 1×10^5 MIA C51 leukemic cells IP and randomly divided into four groups. All rats were treated 6 hours after cell injection for a total of seven consecutive days. Group I received 0.1 ml of buffer; group II, ARA-C (20 mg/kg per day); group III, ImuVert (25 μg/day); and group IV, ARA-C (20 mg/kg per day) and ImuVert (25 μg/day). Alopecia data were recorded on day 9 of the experiment (2 days after ARA-C treatment was stopped). The incidence of death from chloroleukemia in the four groups is also shown.

Group	*n*	Alopecia*				Death from chloro-leukemia
		0	1+	2+	3+	
I (Controls)	21	21	0	0	0	21
II (ARA-C)	23	0	3	4	16	21
III (ImuVert)	20	20	0	0	0	11
IV (ARA-C + ImuVert)	20	15	5	0	0	2

*No detectable alopecia, 0; mild alopecia defined as less than 50% hair loss, 1+; moderately severe alopecia with more than 50% hair loss, 2+; and total absence of hair, 3+.

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Table 3. The occurrence of alopecia in rats treated with DX and CTX; effect of ImuVert. In each experiment, 20 8-day-old rats were randomly divided into two groups. In experiment 1, group I received DX (2 mg/kg per day IP) for 7 days, and group II received DX in the same dose plus ImuVert (25 μg/day SC) for 7 days. Data were recorded on day 12 of the experiment (5 days after DX treatment was stopped). All 10 rats in group I developed complete alopecia over the head and proximal neck, while none of the rats in group II developed alopecia (see Fig. 3). In experiment 2, group I received CTX (25 mg/kg IP) as a single dose, and group II received the same dose of CTX plus ImuVert (25 μg/day IP) for 7 days. The severity of alopecia was scored at 7 days as described in the legend to Table 1.

Group	Alopecia			
	0	1+	2+	3+
Experiment 1				
I (DX)	0	0	0	10
II (DX + ImuVert)	10	0	0	0
Experiment 2				
I (CTX)	0	2	4	4
II (CTX + ImuVert)	0	2	4	4

animals in both groups died rapidly after day 15 of the experiment. The apparent lack of protection from CTX-induced alopecia is not readily explained but strongly suggests that this agent causes alopecia by a mechanism distinct from that caused by ARA-C or DX. On the other hand, it is possible that further manipulation with dose and dosage scheduling could bring about protection against CTX-induced alopecia by ImuVert.

The protection from alopecia by ImuVert is of extreme interest with respect to both molecular mechanisms and therapeutic potential. ImuVert is being developed as a therapeutic agent for cancer and is currently in phase II clinical trials for recurrent primary brain tumors (*16, 17*). ImuVert is capable of activating macrophages in vitro, causing the release of a number of cytokines including interleukin-1, tumor necrosis factor, interferon-gamma, prostaglandin E_2, and granulocyte-macrophage colony-stimulating factor (*18*). It also augments the activity of NK cells and cytotoxic T lymphocytes (*19, 20*).

In a recent study we demonstrated the stimulation by ImuVert of a myeloid differentiation-inducing activity by monocytes (*11*). ImuVert was capable of aborting the development of chloroleukemia in 45% of young rats injected with a leukemia cell load (100% of the control animals died from leukemia) (*11*). On the basis of in vitro and in vivo studies, we postulated that ImuVert acted by increasing the level of endogenous differentiation-inducing activity leading to terminal differentiation of the injected leukemic cells (*11*).

The mechanism of protection from chemotherapy-induced alopecia by ImuVert remains uncertain. It could be mediated by a single cytokine or could represent the summation effect of a number of cytokines. It is also likely that ImuVert protection of the hair follicles from ARA-C and DX occurs at some point in the S phase of the cell cycle, since both of these chemotherapeutic agents are cell cycle–specific and exert their action during DNA synthesis. It is of interest in this regard that ImuVert had no apparent protective effect on the chloroleukemia cells. On the contrary, the combination of ARA-C + ImuVert had a synergistic effect in aborting the development of chloroleukemia in the rat model (Table 1). The 45% survival in the ImuVert-alone group confirms our previously published results (*11*).

Whatever the mechanism, the virtually uniform and complete protection by ImuVert from alopecia due to two of the most commonly used chemotherapeutic agents, ARA-C and DX, in the rat model has exciting prospects for preventing alopecia in the clinical setting. The apparent antitumor effect of ImuVert could offer an additional bonus in the cancer patient. Because ImuVert is already in phase II clinical trials, it should be feasible to examine these possibilities in the near future.

Fig. 3. Color photograph of three rats each from the DX-only group (group I) (left) and from the DX-ImuVert combination group (group II) (right) taken on day 12 of the experiment. Treatment schedule was as described in the legend to Table 2. ImuVert provided complete protection against DX-induced alopecia.

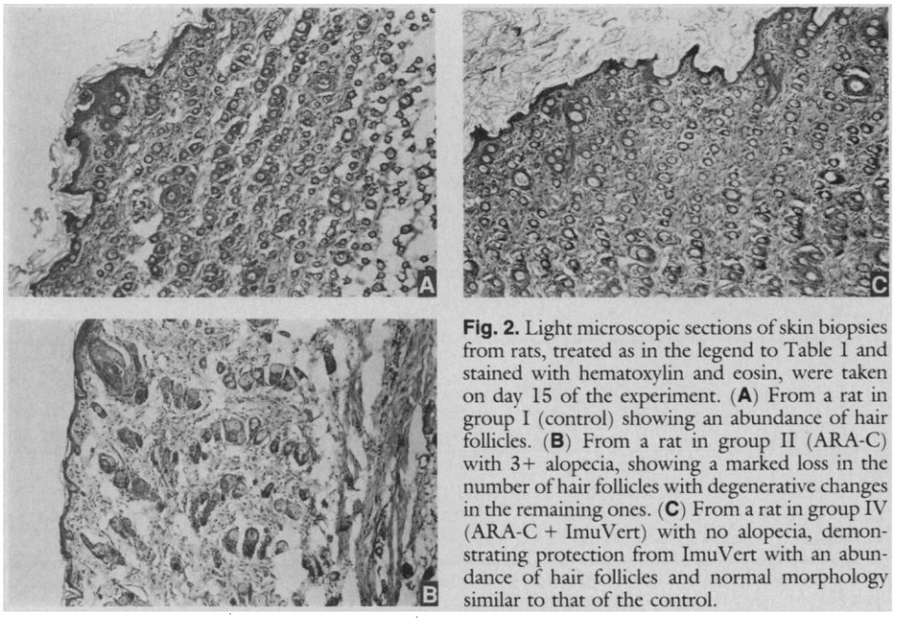

Fig. 2. Light microscopic sections of skin biopsies from rats, treated as in the legend to Table 1 and stained with hematoxylin and eosin, were taken on day 15 of the experiment. (**A**) From a rat in group I (control) showing an abundance of hair follicles. (**B**) From a rat in group II (ARA-C) with 3+ alopecia, showing a marked loss in the number of hair follicles with degenerative changes in the remaining ones. (**C**) From a rat in group IV (ARA-C + ImuVert) with no alopecia, demonstrating protection from ImuVert with an abundance of hair follicles and normal morphology similar to that of the control.

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21. Supported by U.S. PHS grant DK07114 and the Barbara Piasecka Johnson Foundation.

7 March 1990; accepted 12 July 1990