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Combined effects of interferon α and interleukin 2 on the induction of a vascular leak syndrome in mice

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Summary. Immunotherapy with interleukin 2 (IL-2) alone or in combination with lymphokine-activated killer cells can mediate tumor regression in mice and in man. Further dose escalation of IL-2 along with lymphokine-activated killer cells has been prevented by the development of a vascular leak syndrome produced by IL-2. Because we have found that interferon α (IFN-α) or tumor necrosis factor (TNF-α) has synergistic antitumor effects when administered together with IL-2, we have tested the vascular leakage induced by these lymphokine combinations. We used a murine model to quantify vascular leakage by measuring the extravasation of ^{125}I-albumin from the intravascular space as well as the wet and dry lung weights after treatment with different cytokines. Cytokines (or Hanks balanced salt solution) were administered to C57BL/6 mice and 4 h after the last injection the vascular leak was quantified. IFN-α alone did not cause extravasation of radiolabel or increase in wet lung weights, though when given in combination with IL-2, significantly greater extravasation ($P < 0.01$) as well as increase in lung water weights ($P < 0.05$) was observed compared to the response in mice treated with IL-2 alone. IFN-α in combination with IL-2 induced significant vascular leakage earlier than the response induced by IL-2 alone. For example treatment with IFN-α and IL-2 induced accumulation of 14674 ± 605 cpm in the lungs at day 1 while IL-2 alone induced 12340 ± 251 cpm. The degree of vascular leakage was highly related to the dose of IFN-α administered along with IL-2 and increased vascular leak syndrome was evident even at low doses (5000 units) of IFN-α. Immunosuppression of mice by pretreatment irradiation (500 rad) markedly decreased the development of vascular leak syndrome induced by IL-2 and IFN-α. Interestingly IFN-γ and TNF-α did not induce vascular leakage in the lungs when given alone, and did not add or synergize with IL-2 in causing the syndrome. Thus the administration of IFN-α in combination with IL-2 produces a dose-limiting vascular leakage that is more severe than that caused by IL-2 alone, and may be mediated, directly or indirectly by host radiosensitive cells.

Abbreviations used: LAK, lymphokine-activated killer; IFN, interferon; TNF, tumor necrosis factor; IL-2, interleukin-2

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Introduction

The administration of recombinant IL-2, either alone or in conjunction with the adoptive transfer of lymphokine-activated killer (LAK) cells, can mediate the regression of established visceral metastases from a variety of tumors in both mice and man [26, 31, 33, 34, 42–44]. In animal models it has been shown that the efficacy of this immunotherapy is directly related to the dose and duration of the IL-2 administered. The amount of IL-2 that can be given, however, is limited by toxicity especially by an IL-2-induced increase in capillary permeability [16, 28, 45], which causes a vascular leak syndrome. This increase in vascular permeability is also mediated by LAK cells and this phenomenon is dependent on the dose of IL-2 and the number of LAK cells administered [16]. Using a murine model, we have previously studied the increase in vascular permeability, induced by IL-2 alone or by LAK cells and IL-2, by quantifying the extravasation of i.v. injected ^{125}I-albumin [16, 45] into tissues.

IFN-α has also been shown to possess a variety of antitumor effects both in animal tumor models [7, 20, 27] and in clinical trials against some human malignancies [38]. The mechanism of the antitumor effect of IFN-α is unknown but may be related to the direct inhibition of tumor growth [10, 17, 36] or to an indirect action(s), possibly through the augmentation of natural killer activity [7, 8, 20, 27], or to enhancement of the expression of surface molecules including β-2 macroglobulin, Fc receptors, tumor antigens, and histocompatibility antigens [1, 22, 54, 55].

We and others have recently shown that the combined administration of IFN-α and IL-2 mediates a synergistic therapeutic effect in causing the regression of established metastases [9, 11, 37]. TNF-α and IFN-γ have also been shown to possess a variety of antitumor effects [2, 3, 12, 13] and TNF-α and IL-2 synergize in causing the regression of established subcutaneous and hepatic metastases [32]. Because of the widespread interest in performing clinical trials of combinations of cytokines including IFN-α, IL-2, TNF-α, and IFN-γ in cancer-bearing patients, we have studied the vascular leak syndrome induced by these treatments. In this manuscript we demonstrate that IFN-α and IL-2 combined induce this syndrome in the lungs of mice to a greater extent than does either one alone. IFN-γ and TNF-α did not induce vascular leakage alone nor do they synergize with IL-2 in inducing the syndrome.

Material and methods

Mice. Female C57BL/6 mice, 12 weeks old, were obtained from the animal production colonies of the National Institutes of Health (Bethesda, Md). Caged in groups of six or fewer, the animals were fed NIH laboratory chow and were given water ad libitum.

Recombinant cytokines. Human recombinant IL-2, used in these experiments, was kindly provided by Cetus Corporation, Emeryville, California. Purified IL-2 was generated from *Escherichia coli* and had a specific activity of (3–4) $\times 10^6$ U/mg protein [41]. The endotoxin level in the purified IL-2 preparation was <0.1 ng/10^6 U recombinant IL-2 as measured by a standard *Limulus* assay.

Recombinant human TNF-α (rhTNF-α) was also kindly provided by Cetus Corporation, Emeryville, Calif. It had a specific activity 2.2×10^7 U/mg protein and an endotoxin level of 0.097 ng/mg protein.

Recombinant murine IFN-γ (rmIFN-γ) was kindly provided by Genentech Inc. (San Francisco, Calif). It had a specific activity of 0.9×10^7 U/mg protein and an endotoxin level less than 0.034 U/mg.

Recombinant human IFN-α A/D (rhIFN-α A/D) was kindly provided by Hoffmann La Roche Inc. (Nutley, NJ) and was produced from hybrid plasmid. The specific activity of rhIFN-α A/D, which is active on murine cells [6, 40], was in the range (6–10) $\times 10^4$ U/mg protein as assayed by inhibition of the cytopathic effect of vesicular stomatitis virus on murine L cells [46]. All cytokines were diluted in Hanks balanced salt solution (HBSS) containing 0.2% ovalbumin (Sigma Chemicals, St. Louis, Miss).

Measurement of vascular permeability. This assay was performed as previously described by Rosenstein et al. [45] and Ettinghausen et al. [16]. Briefly, mice received intraperitoneal injections of either HBSS alone, IL-2 alone, IFN-α alone or a combination of these cytokines twice on the first day and thrice daily for the subsequent 2 days, and on day 4 one more injection was given in the morning (thus making a total of nine injections). IFN-γ with or without IL-2 was also injected i.p. twice on the first day, thrice daily for the next 2 days and one injection was given on day 4. rhTNF-α was administered i.v. and 2 h later either HBSS or IL-2 was given i.p. in these mice. In some experiments, mice were pretreated with whole-body irradiation (500 rad from a ^{137}Cs source; Gamma cell-40, Atomic Energy of Canada Ltd). On day 4, 0.5 μCi ^{125}I-labeled bovine serum albumin (specific activity 1–2 μCi/μg; New England Nuclear, Boston, Mass) was injected i.v. 4 h after the last cytokine injection in 0.5 ml HBSS containing 1% mouse serum. Mice were sacrificed 1 h later and lungs were harvested, rinsed with water thoroughly and subsequently blotted and counted in a gamma analyzer. A permeability index was then calculated by dividing the mean ^{125}I (cpm) content of tissues from treated mice by the ^{125}I in animals treated only with HBSS. Each treatment group contained four mice. In some experiments the lungs were weighed after counting their radioactivity to determine the increase in lung water weight. Dry weights of lungs were measured after overnight lyophilization of the tissues.

Statistical analysis. The significance of difference in mean radioactivity (cpm) and weight of the lungs between the treatment groups was analysed by using Student's *t*-test. All *P* values are presented as two-sided determinations.

Results

Combined effect of IFN-α on IL-2-induced extravasation of ^{125}I-albumin into lungs

To study the effect of IFN-α and IL-2 on fluid and colloid extravasation from the intravascular space into the lung interstitium, mice received either HBSS alone, 100000 U IFN-α alone, 100000 U IL-2 alone or a mixture of IFN-α and IL-2 (100000 U each) i.p. two times on the first day, thrice daily for the next 2 days and once on day 4. Four hours after the last injection, 0.5 μCi ^{125}I-albumin was injected i.v. into all mice and, 1 h later, lungs were harvested and analyzed in a gamma counter. As shown in two representative experiments in Fig. 1, IL-2 alone caused a significant increase ($P < 0.01$) in extravasation of ^{125}I-albumin compared to the HBSS-treated control group (7910±634 cpm versus 15717±1372 cpm for the HBSS-

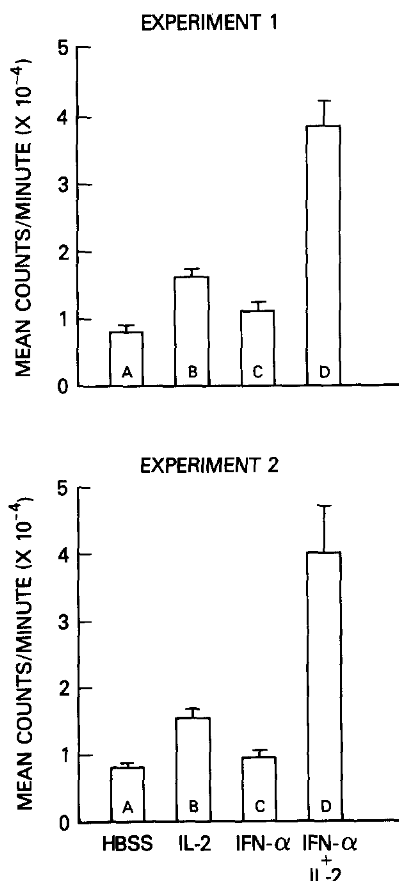

Fig. 1. IFN-α and IL-2-induced extravasation of ^{125}I-albumin in the lungs of mice. C57BL/6 mice were treated with either HBSS alone, 100000 U IL-2 alone, 100000 U IFN-α alone or a mixture of IFN-α and IL-2 i.p. twice on the first day and thrice daily on the subsequent 2 days. On day 4, ^{125}I-albumin (0.5 μCi) was injected i.v. 4 h after the last injection of cytokines, and 1 h later lungs were counted for gamma irradiation. The mean radioactivity ± SEM (cpm) for the lungs from two separate experiments from four mice per treatment group are shown. Mean amount of ^{125}I injected in Exp. 1 was 677017+5457 cpm and 633687±17966 cpm in Exp. 2. Statistical analysis: Exp. 1, A vs B, $P < 0.01$; A vs D, $P < 0.001$; A vs C, $P > 0.05$; B vs D, $P < 0.005$; C vs D, $P < 0.001$

and IL-2-treated groups respectively in experiment 1). IFN-α alone did not cause a significant increase in extravasation, though when given in combination with IL-2 a significant increase in vascular permeability was seen (in experiment 1 38414±3289 cpm was observed in the lungs treated with IFN-α and IL-2, $P < 0.005$, compared to results with IL-2 alone or HBSS-treated control lungs). Similar results were seen in six additional experiments.

To demonstrate that measurement of ^{125}I-albumin extravasation actually reflected fluid accumulation in lung tissues, wet and dry weights of lungs were measured after mice had been treated with HBSS alone, IL-2 alone (100000 U), IFN-α (100000 U) alone or a mixture of IL-2 and IFN-α as described above. A representative experiment is shown in Table 1. Administration of IL-2 alone or IFN-α alone did not cause significant accumulation of fluid in the lungs in this experiment; however, when administered in combination a significant accumulation of water was noted ($P < 0.02$). The dry weight of lungs was not significantly different between any of the groups. In some experiments, an increased accumulation of water in the lungs of mice treated with IL-2 three times a day for 3 days was demonstrated (see Fig. 3).

Kinetics of IL-2- and IFN-α-induced extravasation of ^{125}I-albumin into lungs

In order to demonstrate the time course of fluid and protein extravasation from the intravascular space into the lung interstitium, mice were injected with HBSS alone, IL-2 (100000 U) alone, IFN-α (100000 U) alone or with a mixture of IL-2 and IFN-α (100000 U each) thrice daily for 3 consecutive days. The ^{125}I-albumin extravasation assay was performed on day 4.

As shown in an experiment in Table 2, IL-2 alone caused a significant accumulation of ^{125}I-albumin in the lung interstitium at day 3 ($P < 0.02$). IFN-α alone did not cause an increase in the extravasation of radiolabel except that on day 3 a significant increase in radioactivity was observed. In seven other experiments (not shown) IFN-α alone failed to generate any significant vascular leak after 3 days of treatment. However, when IL-2 was injected along with IFN-α a significant increase in the permeability index was observed starting from day 1, which continued to increase in a time-dependent fashion. The permeability index increased to 1.25 by day 1, 1.75 by day 2 and 3.72 by day 3 (all $P < 0.02$ compared to IL-2 alone).

Table 1. Effect of cytokine treatment on wet and dry lung weights[a]

Treatment	Lung weight[b] ± sem (g)	
	Wet weight	Dry weight
HBSS	0.253±0.018	0.078±0.012
IL-2	0.277±0.011	0.066±0.008
IFN-α	0.242±0.009	0.075±0.011
IL-2+IFN-α	0.342±0.019[c]	0.085±0.009

[a] 100000 U IL-2 alone, IFN-α alone, or a mixture of IFN-α and IL-2 injected i.p. thrice daily for 3 days. On day 4, all animals were sacrified and tissues harvested, weighed and lyophilysed overnight
[b] Mean lung weight for four mice per group
[c] $P < 0.02$ compared to HBSS and IL-2 groups

Table 2. Kinetics of IL-2 and IFN-α-induced extravasation of ^{125}I-albumin in mouse lungs[a]

Treatment	^{125}I assayed ± SEM (cpm) after cytokine administration for		
	1 day	2 days	3 days
HBSS	11744±526	11289±1102	10735± 280
IL-2	12340±251 (1.05)[b]	12436±463 (1.10)	14877± 658[d] (1.39)
IFN-α	13796±896 (1.17)	13128±396 (1.16)	13028± 774[d] (1.21)
IL-2+IFN-α	14674±605[c,e] (1.25)	19797±148[c] (1.75)	39940±5813[c] (3.72)

[a] HBSS, IL-2 (100000 U) IFN-α (100000 U) or IL-2 and IFN-α (100000 U each) were injected i.p. three times a day for the number of days indicated. Results show means ± SEM for four mice per group. Mean injected ^{125}I-albumin was 842176 ± 1612 cpm
[b] Results in parenthesis show the permeability index, i.e. the mean ^{125}I of lungs from mice treated with IL-2 or IFN-α and IFN-α plus IL-2 divided by the mean value of tissues from HBSS-treated animals
[c] $P < 0.02$ versus IL-2-treated group in respective columns
[d] $P < 0.02$ versus HBSS-treated group
[e] NS versus IFN-α-treated group

Dose titration of IFN-α- and IL-2-induced vascular leakage

We next investigated the dose-response relationship between administration of IL-2 and IFN-α – and fluid egress into lung tissues. Mice were given either HBSS alone, IL-2 alone (100000 U), IFN-α alone (100000 U) or a combination of the two, keeping IL-2 constant at 100000 U per injection. IFN-α was varied from 200 U to 100000 U per injection. These injections were given i.p. twice on the first day, thrice on the next 2 days and once on day 4. Four hours after the last cytokine injection ^{125}I-albumin was injected i.v. and lungs were harvested and counted. Figure 2 shows the relationship between the dose of IFN-α and ^{125}I-albumin extravasation in lung tissues. At 5000–100000 U IFN-α along with the combination of 100000 U IL-2, lungs demonstrated a significant increase in radiolabel accumulation compared to IL-2 alone or IFN-α alone ($P < 0.05–0.005$). With 200 U and 1000 U IFN-α the lungs showed only a negligble increase in vascular leakage over the group treated with IL-2 alone ($P > 0.05$).

In similar experiments the wet weights of lungs were determined after treatment. As shown in Fig. 3, IL-2 alone caused a significant increase ($P < 0.0002$) in wet lung weight compared to the HBSS-treated control. IFN-α alone did not increase lung weight; however, when given in combination with IL-2 a significant increase in wet lung weights was seen compared to the IL-2-treated lung. For example, IFN-α at 5000–100000 U along with IL-2 caused a significant increase in lung weights compared to IL-2 alone ($P < 0.05–0.01$) though a uniform dose-response variation was not observed. However, as with ^{125}I-albumin extravasation, IFN-α, at a combination of 1000 U IFN-α and 100000 U IL-2, did not cause an increase in lung water weight compared to IL-2 alone [0.289±0.024 g lung weight was observed in lungs treated with IFN-α (1000 U) plus IL-2 (100000 U) compared to lungs treated with 0.271±0.008 g IL-2 alone].

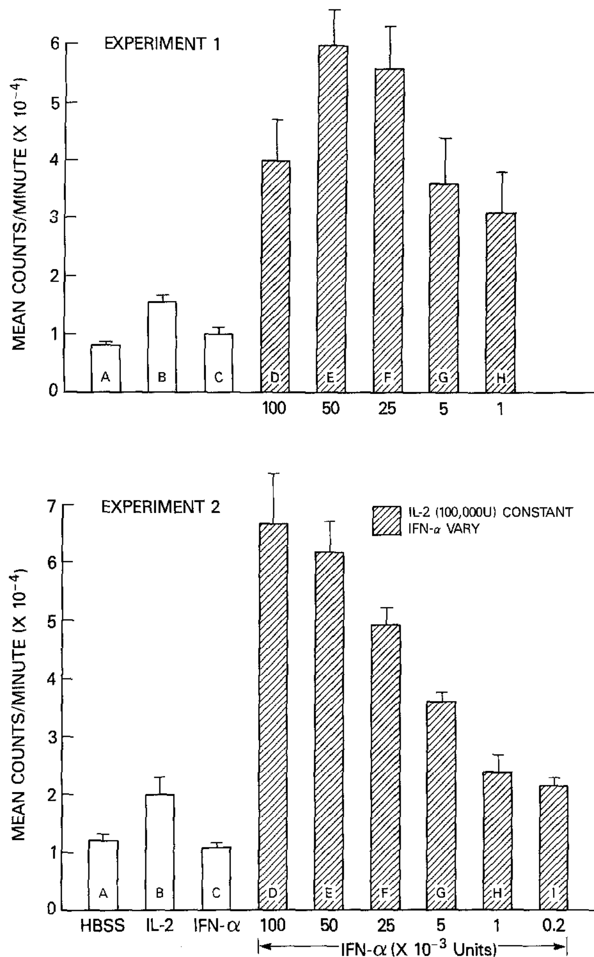

Fig. 2. Dose titration effect of IFN-α and IL-2-induced vascular leak in lungs. C57BL/6 mice were administered either IL-2 alone 100000 U (or HBSS) or IFN-α alone (100000 U) or a mixture of IFN-α and IL-2 twice on the first day and thrice on the next 2 days. The IL-2 dose was constant at 100000 U and the IFN-α dose was varied from 100000 U to 200 U per injection. On day 4 the ^{125}I-extravasation assay was performed 4 h after the last cytokine injection, as described in Materials and methods. Mean radioactivities ± SEM (cpm) are shown from two different experiments. Mean total amount of ^{125}I injected in Expt. 1 was 633687 ± 17966 cpm and 811184 ± 3538 cpm in Expt. 2. Statistical analyses for Expt. 1: A vs B, $P < 0.002$; A vs C, $P > 0.05$; B vs D, $P < 0.01$; B vs E, $P < 0.005$; B vs F, $P < 0.005$; B vs G, $P < 0.05$; B vs H, $P > 0.05$. For Expt. 2: A vs B, $P < 0.02$; A vs C, $P > 0.05$; B vs C, $P < 0.02$; B vs D–G, $P < 0.02$; D vs E and F, $P > 0.05$; D vs G, H and I, $P < 0.05$

Abrogation of IL-2 and IFN-α-induced fluid extravasation

We tested the effect of immune suppression by pre-irradiation of mice on the vascular leak phenomenon. Normal or pre-irradiated mice (500 rad) were treated with either HBSS alone, 100000 U IL-2 alone or a mixture (100000 U each) of IL-2 and IFN-α i.p. twice on the first day and thrice per day for the next 2 days. On day 4, the ^{125}I-albumin extravasation assay was performed 4 h after the last injection of cytokines. In normal mice, IL-2 alone caused a significant increase in ^{125}I-albumin accumulation. IFN-α, when administered with IL-2, caused pronounced leakage beyond that observed with IL-2 alone (Fig. 4). For example, 59464 ± 8893 cpm were observed in IL-2 and IFN-α-treated lungs compared to 20799 ± 994 cpm in IL-2-treated lungs ($P < 0.02$). In irradiated mice the differences between controls and lungs treated with either IL-2 or IL-2 and IFN-α were eliminated (14429 ± 1507 cpm, 14742 ± 1835 cpm and 17218 ± 2420 cpm were observed in

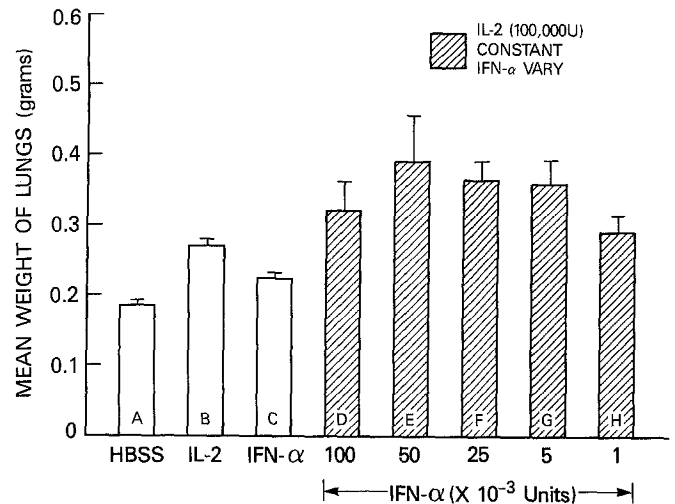

Fig. 3. Pulmonary edema formation induced by IFN-α and IL-2. As described in Fig. 2, C57BL/6 mice were injected with either IL-2 alone (or HBSS), IFN-α alone or a mixture of IFN-α and IL-2 from days 1–4. On day 4 extravasation of ^{125}I-albumin was assayed and then wet lung weights were measured. Mean wet weights of lung ± SEM (g) from four mice per treatment group are shown. Statistical analysis: A vs B $P < 0.0002$; A vs C, $P > 0.05$; B vs D, $P > 0.05$; B vs E, $P < 0.05$; B vs F, $P < 0.01$; B vs G, $P < 0.05$; B vs H, $P > 0.05$

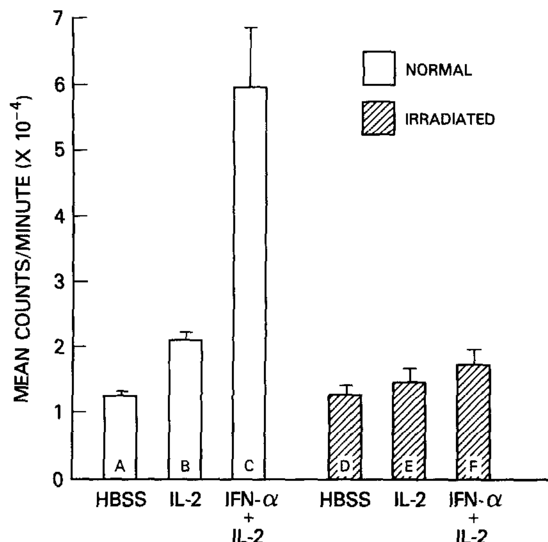

Fig. 4. Abrogation of IL-2 and IFN-α-induced extravasation of intravascular ^{125}I-albumin in lungs of mice by irradiation. Half of the mice received 500 rad of whole-body irradiation and 2 h later i.p. injections of HBSS or 100000 U IL-2 or the combination of 100000 U each of IL-2 and IFN-α were begun and continued for 4 days. On day 4, the ^{125}I-albumin extravasation assay was performed 4 h after last cytokine injection as described in Materials and methods. Means ± SEM (cpm) from four mice per treatment group are shown. Statistical analysis for normal mice: A vs B, $P < 0.02$; A vs C, $P < 0.02$; B vs C, $P < 0.02$. For irradiated mice: D vs E, $P > 0.05$; D vs F, $P < 0.05$; E vs F, $P > 0.05$; B vs E, $P < 0.05$ and C vs F, $P < 0.02$

lungs treated with HBSS, IL-2 and IFN-α plus IL-2 respectively).

Effect of IFN-γ on the extravasation ^{125}I-albumin in the lungs

To evaluate the effect of IFN-γ in inducing vascular permeability with or without IL-2, mice were injected i.p. with HBSS alone, 50000 U IFN-γ alone, 50000 U IL-2 alone or a mixture of IFN-γ and IL-2 from day 1 through day 4 (two injections on the first day, three on each of the subsequent 2 days and one on day 4). On day 4 the extravasation assay was performed 4 h after the last injection of cytokines. As seen in Fig. 5, IL-2 alone caused a significant leakage compared to the control ($P < 0.02$). IFN-γ alone did not affect vascular permeability, nor did it combine with IL-2 to induce the vascular leak syndrome. For example, 15424±454 cpm was observed in IL-2-treated lungs compared to 15165±1522 cpm in lungs treated with IL-2 and IFN-γ ($P > 0.05$). However, in the same experiment, IFN-α combined with IL-2 to cause significant fluid accumulation ($P < 0.0001$). In another experiment higher doses (100000 U per injection, total of nine injections) of IFN-γ were tested with or without IL-2. IFN-γ alone did not cause significant increase in the vascular leak in the lungs of mice. In combination with IL-2, IFN-γ was lethal and 75% of mice died before the extravasation assay.

Effect of rhTNF-α on vascular leakage

rhTNF-α is produced predominantly by monocyte/macrophages [29] and causes biological effects on two likely target tissues involved in acute lung injury – circulating granulocytes [18] and the endothelium [5, 35, 47]. One sublethal dose of rhTNF-α alone (10 μg diluted in HBSS containing 1% mouse serum) was injected i.v. into normal mice. Mice were randomized 2–4 h following hTNF injection to receive either HBSS or 50000 U or 100000 U IL-2 i.p. three times a day from day 1 through day 4. On day 4 the extravasation assay was performed 4 h after the last injection. Fig. 6 shows that rhTNF-α alone did not increase extravasation of ^{125}I-albumin in the lungs (9331±430 cpm were obtained in rhTNF-α-treated lungs compared to 8398±357 cpm in HBSS-treated lungs, $P > 0.05$); however, IL-2 alone caused a significant increase in the ^{125}I-albumin extravasation in the same experiment ($P < 0.002$). Furthermore, when IL-2 was injected into rhTNF-α-treated mice no enhancement in IL-2-induced vascular leakage was observed. A total of 16701±795 cpm was obtained in lungs treated with rhTNF-α and IL-2 compared to 14186±1025 cpm in lungs treated with IL-2 alone ($P > 0.05$). Similar to the results of ^{125}I-albumin extravasation, the wet weights of the lungs were significantly increased ($P < 0.01$) in IL-2-treat-

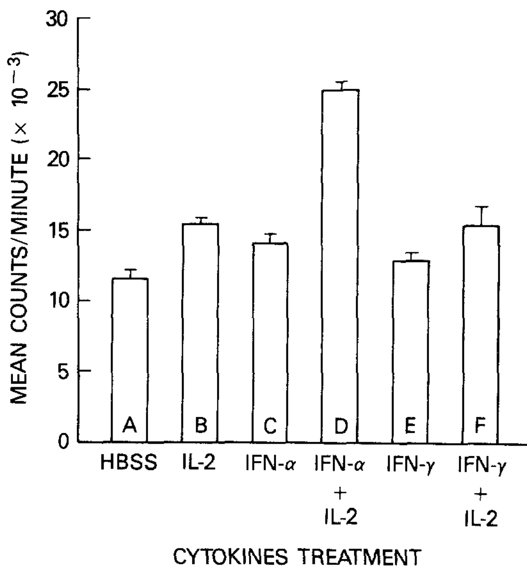

Fig. 5. Effect of IFN-γ on vascular permeability in the lungs of mice. 50000 U IL-2 alone (or HBSS) or IFN-γ alone, IFN-α alone or combinations of IFN-γ and IL-2 or IFN-α and IL-2 were injected i.p. in C57BL/6 mice from days 1–4. On day 4 the ^{125}I-albumin extravasation assay was performed as described in Materials and Methods. Means from three or four mice per group ± SEM (cpm) are shown. Total mean radioactivity injected was 947728±1386 cpm (± SEM). Statistical analyses: A vs B, $P < 0.02$; A vs C, $P > 0.05$; B vs D, $P < 0.0001$; A vs E, $P > 0.05$; A vs F, $P > 0.05$; B vs F, $P > 0.05$

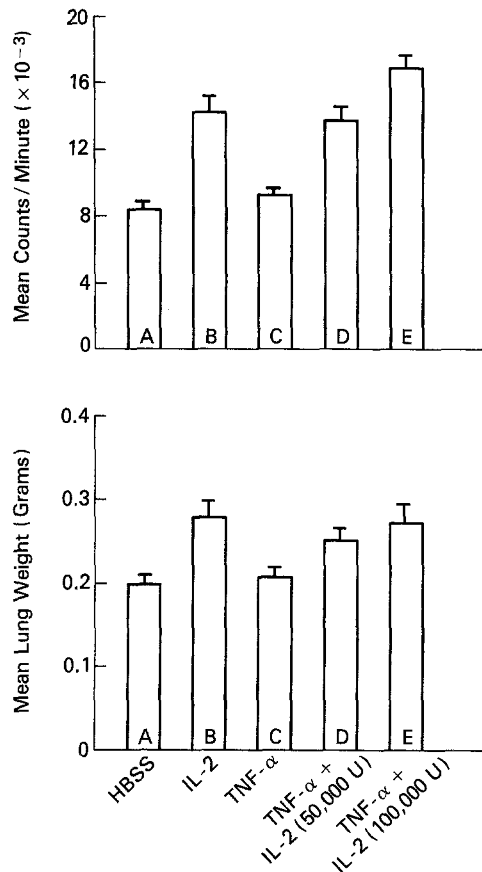

Fig. 6. Effect of TNF-α on the extravasation of ^{125}I-albumin and pulmonary edema formation in the lungs of mice. C57BL/6 mice were administered either HBSS or 100000 U IL-2 i.p. three times a day for 3 days. TNF-α (10 μg) was given i.v. on day 0 and 2 h later mice were randomized to receive either HBSS or 50000 U or 100000 U IL-2 thrice daily for 3 days. On day 4 the ^{125}I-albumin extravasation assay was performed. Wet lung weights were merasured and gamma irradiation was measured. Mean radioactivity injected 765532±12065 cpm *Upper Panel*, mean ± SEM (cpm): A vs B, $P < 0.02$; A vs C, $P > 0.05$; B vs C, $P < 0.02$; C vs D and E, $P < 0.05$. *Lower panel*, mean wet lung weight ± SEM (g): A vs B, $P < 0.01$; A vs C, $P > 0.05$; A vs D, $P < 0.02$; A vs E, $P < 0.05$; B vs C, $P < 0.05$; B vs D and E, $P > 0.05$

ed mice but not in mice treated with TNF-α alone. The wet weights of the lungs were also increased significantly ($P < 0.02$) in mice treated with rhTNF-α plus IL-2 but not compared to mice treated with IL-2 alone ($P > 0.05$).

In another experiment, the ^{125}I-albumin extravasation assay was performed in animals 4 h and 24 h after i.v. injection of either 5 μg or 10 μg rhTNF-α. No significant increase in ^{125}I-albumin extravasation was observed at either of these times compared to HBSS-treated control lungs (data not shown). rhTNF-α (8 μg) was also given as one daily dose (i.v.) for 3 days with or without IL-2. Treatment with rhTNF-α alone failed to cause any increase in the accumulation of ^{125}I-albumin in the lungs. A total of 3569 ± 79 cpm was observed in rhTNF-α-treated lungs compared to 3465 ± 429 cpm in HBSS-treated control. The TNF-α and IL-2 combination was too toxic and all mice died prior to the vascular leak assay. A group of mice treated with IL-2 was included in all of these experiments as a positive control and a significant increase in the accumulation of radiolabeled albumin was observed over HBSS-treated controls (data not shown).

Discussion

Adoptive immunotherapy with LAK cells and IL-2 can result in significant regression of visceral metastases in mice as well as in man [26, 31, 33, 34, 42–44]. IL-2, however, induces a vascular leak syndrome that limits the amount of IL-2 that can be administered [16, 28, 45]. We have previously shown that the syndrome is mediated at least in part by Thy-1.2$^-$, L3T4$^-$, Lyt-2$^-$ and asialo-GM-1 (ASGM-1)$^+$ precursor cells, which directly or indirectly produce vascular leakage when given with IL-2 [16]. The ASGM-1 cell-surface marker has been found on a majority of natural killer and LAK cell precursors [24, 25, 58] however, some cytotoxic T-lymphocyte precursors also bear this antigen [52].

In a variety of animal tumor models the combined administration of either IFN-α or TNF-α along with IL-2 produces enhanced or synergistic antitumor effects [11, 37, 32]. We have therefore investigated the effect of combined cytokine administration in the vascular leak syndrome. The extravasation of radiolabeled albumin into tissues provides an assay of the vascular leak that correlates well with changes in the wet weights of the tissues, and we have therefore used these assays in the present study [45]. In this paper we administered IFN-α, IFN-γ and TNF-α, either alone or in combination with IL-2, to normal mice and found that none of these cytokines alone, with the exception of IL-2, induced vascular leakage. However, IFN-α administered in combination with IL-2, produced enhanced vascular leakage in the lungs. IFN-γ and TNF-α did not synergize with IL-2 in inducing the syndrome.

The mechanism of the enhanced vascular leak syndrome induced by the combined administration of IFN-α and IL-2 is unknown. We previously showed that adoptive transfer of LAK cells along with IL-2, into irradiated mice, increased vascular permeability while either treatment alone in irradiated mice failed to produce the syndrome [16]. We also showed that adoptively transferred LAK cells proliferated in vivo when exogenous IL-2 was administered [15]. It thus appears that these proliferating LAK cells cause an increased vascular permeability either by damaging endothelial cells or by producing a factor(s) in vivo that can cause increased vascular permeability. IFN-α may enhance either or both of these effects. We have searched, but have been unable to identify any factor(s) in LAK cell culture supernatants that mediate increased vascular permeability, as assayed in guinea pig skin using an Evans blue dye extravasation method (Puri and Rosenberg, unpublished data). Others, however, have used this procedure to identify vascular permeability factors in supernatants of human peripheral blood lymphocytes stimulated with conavalin A in vitro [4, 49, 50]. Various tumor cell lines have also been shown to produce factors inducing vascular permeability in culture supernatants [48].

It is possible that IFN-α in combination with IL-2 may well generate "more potent" LAK cells in vivo and thus induce the increased vascular leakage observed in our study. Other intermediate cells may play a role in damaging the vascular endothelium in the lungs. Several reports have indicated that IFN-α can activate human monocytes in vitro and lyse human tumor cells in vitro [23, 53]. IL-2 may also induce tissue monocyte/macrophages to produce oxygen radicals and secrete prostaglandins [14] both of which might affect water and protein transport in the lungs. IFN-γ, however, is a potent activator of macrophages and the inability of IFN-γ to enhance the vascular leakage argues against a major role for macrophages in this phenomenon. Granulocytes have been implicated as effector cells in experimental systems of pulmonary vascular endothelial injury and in the pathogenesis of acute respiratory distress syndrome. Pretreatment granulocyte depletion has been shown to diminish significantly or prevent acute lung injury in response to endotoxin challenge [19, 21] on phorbol myristate acetate administration [51] to rabbits. The role of granulocytes in acute lung injury has been explained through the generation of endothelium-damaging substances including O_2 radicals, proteolytic enzymes, and platelet-activating factor [56, 57]. In other experimental models of acute lung injury, no consistent role for the granulocyte has been demonstrated [39, 57]. Our studies imply that the vascular leak syndrome induced by IFN-α and IL-2 is a lymphoid cell phenomenon, as do our previous results [16, 45], since abrogation of vascular leakage was seen in pre-irradiated mice.

Finally, the combination of IFN-α plus IL-2 appears to exert synergistic therapeutic effects against tumors in a variety of animal tumor models [9, 11, 37, 44a]. It is possible that this increased therapeutic efficacy is related to the increased vascular leak syndrome described in this paper by allowing more liberal egress of immune lymphoid cells from the intravascular space into the interstitium, where tumor cells can be recognized and destroyed.

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