The Rapid Induction by Interleukin-2 of Pulmonary Microvascular Permeability

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The clinical use of interleukin-2 (IL-2) is limited by severe cardiopulmonary dysfunction. This study examines the mechanism of respiratory failure related to IL-2, using sheep with chronic lung lymph fistulae. Awake animals were infused with an intravenous (I.V.) bolus of IL-2 10^5 U/kg (n = 5) or its excipient (EXC) control (n = 3), every 8 hours for 4 to 5 days. Cardiopulmonary function was monitored daily for at least one 8-hour period. Within 2 hours after each IL-2 administration, mean pulmonary arterial pressure (MPAP) rose. On Day 1, the mean rise was from 13 to 26 mmHg ($p < 0.05$), and on Day 5, to 29 mmHg ($p < 0.05$). MPAP returned to baseline levels after 2–3 hours. Pulmonary arterial wedge pressure was unchanged from 4 mmHg. There were transient falls in arterial oxygen tension, from 88 to 77 mmHg on Day 1 and to 73 mmHg ($p < 0.05$) on Day 5. Lung lymph flow ($\dot{Q}L$) rose from 2.4 to 6.8 ml/30 minutes ($p < 0.05$) on Day 1, and from 4.7 to 10.2 ml/30 minutes ($p < 0.05$) on Day 5, whereas the lymph/plasma protein ratio increased on Day 1 from 0.69 to 0.83 ($p < 0.05$) and from 0.63 to 0.71 ($p < 0.05$) on Day 5. This documents an increase in pulmonary microvascular permeability. Thromboxane (Tx)B2 levels increased transiently after each IL-2 injection in plasma from 195 to 340 pg/ml ($p < 0.05$) and in lung lymph from 222 to 772 pg/ml ($p < 0.05$) on Day 1, and to similar levels on Day 5. There was a progressive rise in cardiac output from 5.7 to 8.6 l/minute ($p < 0.05$) during the 5 days of infusion. Systemic blood pressure did not change. Temperature rose from 39.1 to 41.2 C ($p < 0.05$), and shaking chills were common. There was a progressive fall in leukocyte count, from 8.4 to 3.2 $\times$ 10^3/mm^3 ($p < 0.05$) by Day 5, reflecting a 77% fall in lymphocytes. Lung lymph lymphocyte counts rose, and lymphocyte clearance increased. Lung histology revealed lymphocyte sequestration in capillary beds and peribronchial areas. EXC control infusions resulted in mild fever, increase in MPAP from 12 to 17 mmHg ($p < 0.05$), and $\dot{Q}L$ from 2.6 to 5.1 ml/30 minutes, along with a fall in the L/P protein ratio from 0.77 to 0.71 ($p < 0.05$)—changes that did not indicate increased permeability and were noted only on Day 1. The effect of IL-2 on endothelial cell (EC) barrier function was studied directly using bovine aortic EC grown to confluence on microcarrier beads. The EC barrier to the passage of trypan blue conjugated to albumin from the cultured medium into the microcarrier matrix was not altered by IL-2 ($1–10^4$ U/ml) or EXC. These data indicate that IL-2 leads to lung injury manifest by pulmonary hypertension and rapid increase in lung vascular permeability, effects likely mediated by lymphocytes and Tx. IL-2 alone has no direct effect on the vascular barrier.

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ADOPTIVE IMMUNOTHERAPY using IL-2 and lymphokine activated killer (LAK) cells is limited by significant toxicity.[1,2] Respiratory failure with hypoxia, pulmonary edema, and systemic hypotension are the most significant complications encountered, often necessitating cessation of therapy. Further, there is a marked weight gain with generalized edema, thought due to a "vascular leak syndrome" (VLS).[3] Until now, only small animals have been used to study the side effects of IL-2/LAK cell treatment.[3,4] These studies have limitations regarding the interpretation and sequential monitoring of physiologic changes. It has been difficult to conclude whether this VLS reflects an increase in microvascular permeability, and further, whether the changes observed are mediated by IL-2 alone or whether the concomitant administration of LAK cells is required.[3-5] This study examines the pulmonary and systemic consequences of intravenous IL-2, using the sheep lung lymph preparation, the focus being changes in microvascular permeability.

Methods

Sheep Preparation

Female animals (n = 8) weighing 25–38 kg were prepared with a chronic lung lymph fistulae according to a

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modification of the technique described by Staub.[6] During surgery, animals were anesthetized with intravenous (I.V.) pentobarbitol sodium 15 mg/kg, paralyzed with 2 mg pancuronium bromide, intubated, and mechanically ventilated with a volume cycled respirator using room air. Through a right posterolateral thoracotomy, the efferent duct of the caudal mediastinal lymph node was cannulated with a heparinized Silastic™ catheter (No. 602-155, Dow Corning Corp., Midland, MI). The distal portion of the lymph node, just below the level of the inferior pulmonary ligament, was transected and ligated. All visible systemic lymph tributaries to its proximal portion were cauterized or ligated to minimize extra-pulmonary contamination of collected lymph. The thoracotomy was closed and the lymphatic cannula was exteriorized through the chest wall. A thermistor tipped pulmonary arterial (Electro-cath Corp., Rahway, NJ) and a central venous catheter were introduced through the right internal jugular vein. The aorta was cannulated via the adjacent carotid artery. After a recovery period of 4–5 days, when animals appeared vigorous, were afebrile, and had a steady flow of blood-free lymph, the experiment was conducted.

Cardiopulmonary Function

Stain-gauge transducers (model D-240, Bently Laboratories, Inc., Irvine, CA) were used to measure the following pressures: mean arterial (MAP), mean pulmonary arterial (MPAP) and pulmonary arterial wedge (PAWP). The pulmonary microvascular pressure (Pmv) was calculated from the Gaar equation, Pmv= PAWP + 0.4 (MPAP-PAWP).[7] Pulse rate was determined from an arterial pressure trace. Cardiac output was measured in triplicate by thermodilation (Model 5000, Electro-Cath Corp., Rahway, NJ). Blood gases, pH, oxygen saturation, and hemoglobin of arterial and mixed venous blood were measured with Clark and Severinghaus electrodes and by spectrophotometry using extinction coefficients specific to sheep blood (Model 813 and 282, Instrumentation Laboratory, Lexington, MA).

Hematology

Circulating platelets and white blood cells (WBC) were counted by means of phase microscopy. Differential counts were made on Wright's stained blood smears. WBC were counted in lung lymph and multiplied by lymph flow to obtain lymph lymphocyte clearance.

Biochemical Assays

Plasma and lymph concentrations of thromboxane (Tx)B2 and 6-keto-PGF1, the stable hydrolysis products of TxA2 and prostacyclin, were measured in duplicate by radioimmunoassay.[8,9] Blood was drawn into cooled syringes containing ethylene diamine tetracetic acid (EDTA) and aspirin. The blood was immediately centrifuged at 1500 × g at 4 C for 20 minutes, and the plasma was separated and stored at −20 C until assayed.

Lung lymph was collected at 30-minute intervals in cold graduated tubes containing EDTA and aspirin. The lymph was then centrifuged at 1500 × g and 4 C for 20 minutes, and the supernatant was separated and stored at −20 C until assayed for TxB2 and 6-keto-PGF1. Lymph (L) and plasma (P)total protein concentrations were determined in duplicate by the spectrophotometric protein dye method described by Bradford.[10] The L/P protein ratio was calculated and multiplied by lymph flow ($\dot{Q}L$) to obtain the lymph protein clearance.

Histologic Examinations

At the end of the experiment, animals were killed with an overdose of pentobarbitol and potassium chloride. Gluteraldehyde was instillated into the lungs through an endotracheal tube at a pressure of 25 cm H_2O. After 20 minutes, the hilum of the left lung was clamped. The lung was removed and immersed in gluteraldehyde for 72 hours before sampling. Tissue samples were also taken from the heart, liver, spleen, and kidney. All microscopic sections were stained with hematoxylin and eosin and were interpreted by a pathologist in a blinded fashion.

IL-2

The recombinant interleukin-2 (ala-125) and its excipient control (EXC) (vehicle without IL-2) used in these experiments were provided by the Amgen Corporation (Thousand Oaks, CA). The gene for IL-2 was synthesized chemically and inserted into *Escherichia coli*, where it was expressed at high levels.[11] After purification, the material had a specific activity of 2.8×10^6 U/mg protein. IL-2 activity was measured in a standard assay using the CTLL-2 dependent cell line.[12] One unit of IL-2 activity was defined as that quantity which gave half maximum activity in the bioassay. IL-2 and EXC control were identical with respect to the concentration of glucose (5%) and sodium acetate (10 nM). Endotoxin content in the IL-2 was 1.56 ng/mg protein as determined by the limulus amebocyte lysate assay. Because IL-2 was infused in a dose of 10^5 U/kg, the endotoxin content in this dose was 0.06 ng/kg. No protein was contained in the EXC control. Final container testing and cultures were carried out at the Amgen Corporation.

Protocol

Experiments were performed on awake sheep with free access to food and water. They were allowed to stand or recline as desired and tranducers were relevelled as necessary. IL-2 10^5 U/kg (n = 5) or its EXC control (n = 3) were administered as bolus infusions through the central

venous line every 8 hours for 4–5 days. Because one of the EXC-treated sheep was monitored for 4 days only, the results for this group are presented for this time period. Cardiopulmonary function was continuously monitored while blood and lymph were sampled on multiple occasions during at least one 8-hour period each day after IL-2 or EXC administrations.

Animals used in this study were maintained in accordance with the guidelines of the Committee of Animals of the Harvard Medical School and those prepared by the Committee on care and use of laboratory animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW publication No. 78-23, revised 1978).

In Vitro *Studies*

The effect of IL-2 on endothelial cell barrier function was evaluated using the permeability assay originally described by Boiadjieva[13] and modified by Bottaro.[14] This technique allows the quantitation of transendothelial solute transport by measuring the movement of tracer dye conjugated to albumin through a monolayer of endothelial cells grown on microcarrier beads. The movement of the tracer dye from the medium into the bead matrix is followed spectrophotometrically. Bovine aortic endothelial (BAE) cells were obtained as described by Shepro.[15] Cultures were characterized as endothelial based on morphologic and immunologic criteria.[16] Cultures were grown in a 10% carbon dioxide, 90% air atmosphere at 37 C in Delbecco's modification of Eagle medium (DMEM) (Gibco, Grand Island, NJ). This medium was supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), amphotericin B (0.25 μg/ml) (Sigma, St. Louis, MO), L-glutamine (2mM) (Flow Lab, McLean, VA) and 10% fetal calf serum (Hyclone Labs, Logan, VT). Viability of the cells was assessed by differential uptake of ethidium bromide/fluorescein diacetate.[17]

Primary cultures were subcultured a maximum of three times before seeding onto Cytodex 3 microcarrier beads (Pharmacia, Inc., Piscataway, NJ). Microcarriers were kept in suspension using a Techne MCS-104S magnetic stirrer (Techne Inc., Needham, MA) operated at 44 rpm inside a conventional tissue culture incubator. Endothelial cells were seeded onto the microcarriers at a minimum density of ten cells per bead, and allowed to grow at least 6 days postconfluency. At that time, the microcarriers were used for the permeability assay.

Trypan-blue (TB) dye (Fisher, Pittsburg, PA) and bovine serum albumin (BSA) (Sigma) were added to Hank's balanced salt solution (HBSS) buffered with 10 mM N-2-hydroxyethyl piperazine-N-2-ethenesulfonic acid (HEPES, Research Organics Cleveland, OH) containing glucose 3% and adjusted to pH 7. 4, so that the final concentrations of TB and BSA were 0.2% and 0.45%, respectively. The molecular weight of this marker is 100,000.[14] Samples of 4 ml of microcarrier suspension, with an estimated concentration of 30,000 microcarrier/ml were placed in a plastic vial. The growth medium was replaced by HBSS containing 3% glucose and 10 mM HEPES. The microcarriers covered with endothelial cells were incubated with medium into which IL-2 or EXC were added to a concentration of 0 to 10^4 U/ml, or cytochalasin B in a final concentration of 10^{-5}M. The vials were agitated in 37 C water bath. After 1 (n = 5) or 4 (n = 3) hours of incubation, the medium was removed and replaced by the TB-BSA dye solution to which the reagents under study had also been added. The vials were placed in a 37 C water bath and agitated gently. At 5, 15, 30, 45, and 60 minutes, 150 μl aliquots of beads and dye solution were removed from the vials and placed on an oil cushion of dibutyl:dioctyl phthalate in a ratio of 3:1, respectively. The aliquots were then centrifuged for 30 seconds at 1500 × g. This effectively separated the microcarriers from the medium and terminated the dye uptake by the microcarrier beads. Dye concentration in the supernatant was then assayed by mixing a 100 μl aliquot of supernatant with 900 μl of distilled water. Absorbance was read at 580 nm with a Beckman DU-50 spectrophotometer. All samples were performed in triplicate.

Results are expressed as the reciprocal of the supernatant absorbance because the amount of dye that crossed the endothelial barrier and was trapped within the microcarrier matrix varied inversely with the amount remaining in the medium, (*i.e.*, the supernatant). Because barrier integrity is expressed as dye uptake per bead, the total number of beads per sample was measured by assaying the dextran content of the microcarriers.[18] Briefly, 50 μl bead samples were dissolved in 5 ml of 15M H_2SO_4 for 15 minutes, and then 2 ml of 2.25% phenol was added. This produced a colored product that absorbed at 485 nm. Samples were compared with a standard curve of known bead density to assess bead number per vial. Naked microcarriers were included in every experiment to provide an upper limit for the rate of TB-BSA uptake into the microcarrier matrix.

All data are presented in the text and tables as mean ± standard error. Statistics are based on an analysis of variance, paired and nonpaired t-tests. Significance was accepted if $p < 0.05$.

Results

The sheep became ill soon after each IL-2 administration with frequent cough and labored respiration. After 3–5 hours, the sheep again appeared normal. Infusion of EXC led to no adverse clinical effect.

Alterations in hemodynamic, hemotologic parameters, and microvascular permeability occurred at different time

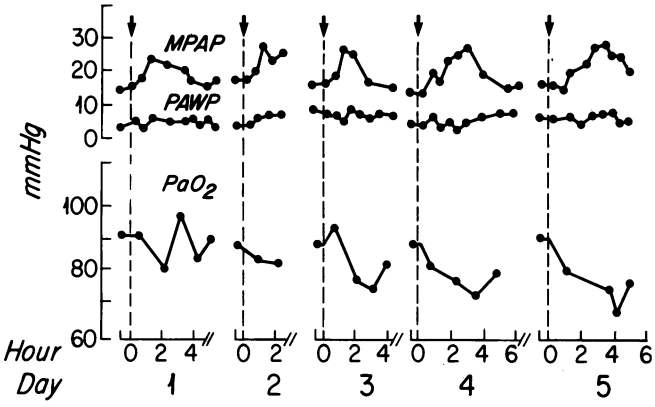


FIG. 1. In a representative animal (Animal 2), each IL-2 injection (arrow) led to transient pulmonary hypertension with an unchanged pulmonary arterial wedge pressure. Progressive hypoxemia developed.

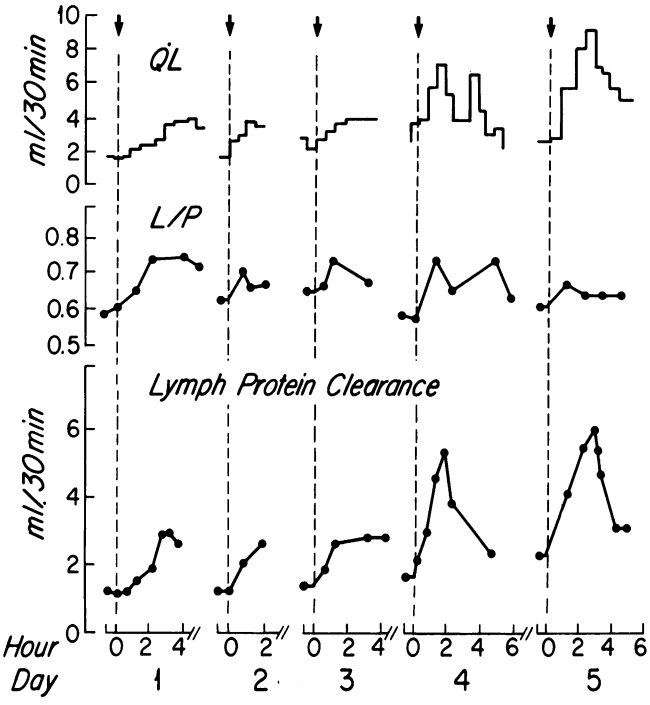


FIG. 2. IL-2 administration (arrows) led to increases in lung lymph flow ($\dot{Q}$), lymph/plasma protein ratio and lymph protein clearance ($\dot{Q} \times L/P$), changes indicating increased pulmonary microvascular permeability. The data is from Animal 2.

intervals after IL-2 administration, but always within 2 hours. For clarity, data in tables are presented as the greatest change. Figures show all results from one animal.

Within 2 hours after each IL-2 infusion, pulmonary hypertension developed and lasted for 1–2 hours (Fig. 1, Table 1). This occurred without an increase in PAWP. At the same time, transient hypoxia occurred that progressed over the 5-day course with an unchanged arterial PCO_2 of 34 ± 3 mmHg. There was a three- to sixfold increase in $\dot{Q}L$ ($p < 0.05$), a significant rise in the L/P protein ratio ($p < 0.05$) and in lymph protein clearance ($p < 0.05$), evidence of a rise in pulmonary microvascular permeability (Fig. 2, Table 1). These changes where noted 2–5 hours after IL-2 infusion and then returned toward baseline. However, baseline was not restored and there was a progressive rise in $\dot{Q}L$ and lymph protein clearance over the 5 days of IL-2 administration.

Increases in plasma and lung lymph TxB2 levels were noted (Fig. 3, Table 1). Plasma TxB_2 concentration peaked within 2 hours of IL-2 injection and declined to baseline levels within 3 hours (Table 1). Levels of 6-keto-PGF1 were unchanged from the baseline value of 25 7 pg/ml.

Cardiac output rose progressively, and by the fifth day, the increase was significant (Fig. 4, Table 2). Aortic pressure tended to decline over the 5 days of IL-2 administration, but this was not significant. Temperature increased transiently after every IL-2 injection, and shaking chills were common (Fig. 4, Table 2).

TABLE 1. *Pulmonary Hemodynamics, Lung Fluid Balance, and Thromboxane Levels After IL-2 and EXC Control Administration*

	Pretreatment	Day 1 IL-2	Day 5 IL-2	Pretreatment	Day 1 EXC	Day 4 EXC
MPAP mmHg	12.9 ± 0.5	25.6 ± 2.8*	29.4 ± 6.1*	11.5 ± 0.4	17 ± 1.5*†	14.0 ± 0.5†
Pmv mmHg	7.5 ± 0.6	13.3 ± 1.9*	14.7 ± 1.7*	7.1 ± 0.7	9.3 ± 1	8.2 ± 0.6†
PAWP mmHg	3.9 ± 0.6	4.9 ± 0.9	4.8 ± 0.9	4.1 ± 0.7	4.3 ± 0.8	4.4 ± 0.6
PaO2 mmHg	88 ± 3	77 ± 3	73 ± 3*	93 ± 1	84 ± 1	84 ± 3†
$\dot{Q}L$ ml/30 minutes	2.4 ± 0.3	6.8 ± 0.1*	10.2 ± 0.6*‡	2.6 ± 0.4	5.1 ± 1.7	4.0 ± 1.6†
L/P	0.69 ± 0.02	0.83 ± 0.03*	0.71 ± 0.03*§	0.77 ± 0.02	0.71 ± 0.02*†	0.67 ± 0.02
Lymph protein clearance ml/30 minutes	1.6 ± 0.2	4.7 ± 0.7*	6.3 ± 0.5*‖	2.1 ± 0.4	3.4 ± 0.5†	2.6 ± 0.3†
Plasma TxB2 pg/ml	195 ± 40	340 ± 24*	776 ± 367*	125 ± 62	276 ± 67	253 ± 22†
Lymph TxB2 pg/ml	222 ± 30	772 ± 159*	706 ± 35*	251 ± 11	333 ± 58†	260 ± 22†

* $p < 0.05$ compared with pretreatment values.

† $p < 0.05$ between groups.

‡ The pretreatment value on Day 5 of 4.7 ± 0.8 was significantly higher than baseline value on Day 1.

§ The pretreatment values of .63 ± .01 was significantly lower than 0.71 ± .03.

‖ Pretreatment value of 2.7 ± 0.5 on Day 5 was higher than baseline value on Day 1.

These were the only substantially different pretreatment values compared with the pretreatment values of Day 1.

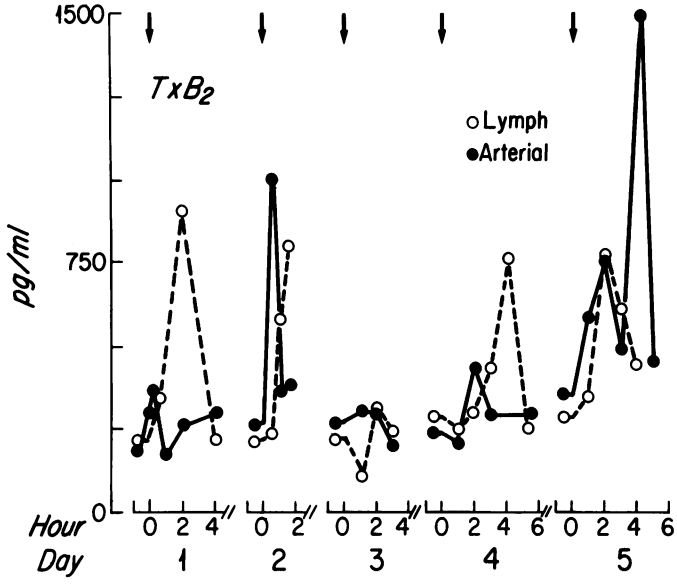


FIG. 3. IL-2 injections (arrows) led to a rise in thromboxane (Tx) B_2 levels in both plasma and lung lymph. The data from Animal 2 is shown.

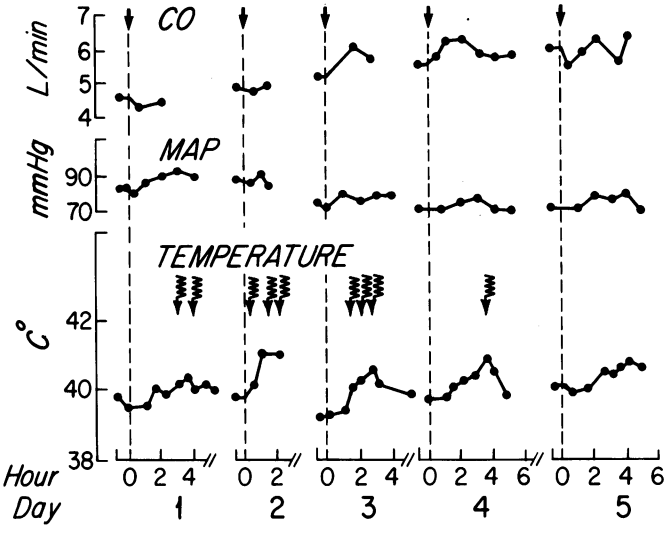


FIG. 4. IL-2 induced a sustained rise in cardiac output (CO) and a downward trend in arterial pressure (MAP). Fever and shaking chills (coiled arrows) were noted. The data from Animal 2 is shown.

On the first day, EXC control infusions resulted in a rise in temperature, mild pulmonary hypertension, and an increase in $\dot{Q}L$ (Table 2), along with a decrease in L/P protein ratio, events not noted subsequently (Table 1). EXC injection did not affect PaO_2, prostanoid levels, or systemic hemodynamics (Tables 1 and 2).

Each IL-2 administration led to a stepwise fall in platelet count ($p < 0.05$), resulting in a decrease to 42,000/mm^3 (Table 3). There was also a progressive fall in WBC count ($p < 0.05$) over the 5 days of IL-2 treatment (Table 3). This leukopenia reflected mainly a decline in the number of lymphocytes that reached 23% of their baseline value ($p < 0.05$). In addition, there was a moderate decrease in the granulocyte count while monocytosis and eosinophilia developed.

Differential counts of leukocytes in lung lymph showed that lymphocytes comprised 98–99% of all cells. From Day 3 and thereafter, IL-2 led to a progressive increase in lymph lymphocyte numbers and clearance ($p < 0.05$) (Fig. 5, Table 3). EXC control injections did not induce significant hematologic change (Table 3).

Macroscopically, the lungs appeared normal after 4 or 5 days of IL-2 or EXC administrations. Histologic examination of the lungs of IL-2-treated sheep showed a diffuse and marked lymphocytic infiltrate around airways and blood vessels (Fig. 6). Lymphocytes were also prominently sequestered within alveolar capillaries (Fig. 7), and occasionally completely occluded small arteriols. Areas of pulmonary granulocyte accumulation were also found. The alveolar spaces and bronchial lumena were histologically normal. Lymphosequestration, although less prominent, was also observed in capillaries and/or perivascular spaces of the liver, spleen, and kidney. Specimens from EXC-treated sheep showed normal histology (Figs. 6 and 7).

Bovine aortic EC morphology and viability were unaltered by a 4-hour incubation with IL-2 or EXC at concentrations up to 10^4 U/ml. The movement of TB-BSA into the microcarrier matrix was significantly reduced by adding a monolayer of EC cells on the microcarrier surface (Fig. 8). The amount of TB-BSA taken up by the EC-covered microcarriers reached a steady state between 45 and 60 minutes after exposure to the tracer dye and was typically less than 50% the amount taken up by the naked microcarriers. Treatment of EC-covered microcarriers with IL-2 or EXC for 1–4 hours at concentrations of 1–

TABLE 2. *Systemic Changes Induced by IL-2 or its EXC Control*

	Pretreatment	Day 1 IL-2	Day 5 IL-2	Pretreatment	Day 1 EXC	Day 4 EXC
CO l/minute	5.7 ± 0.4	6.8 ± 1.2	8.6 ± 1.1*	6.5 ± 1	6.4 ± 0.9	7.2 ± 1.2
MAP mmHg	85 ± 6	81 5 ± 5	75 ± 6	88 ± 4	82 ± 6	83 ± 6
Temperature C	39.1 ± 0.2	41.2 ± 0.1*	40.5 ± 0.5*	39.2 ± 0.3	40.3 ± 0.3*†	39.9 ± 0.3

* $p < 0.05$ *vs.* baseline values.

† $p < 0.05$ between groups.

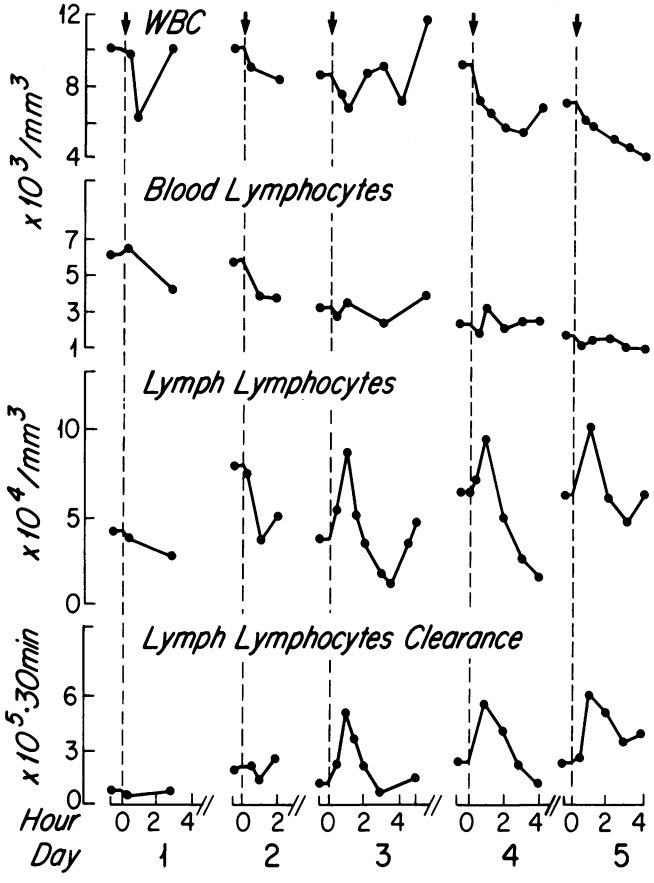


FIG. 5. As illustrated by Animal 2, IL-2 administration (arrow) induced leukopenia, which mainly reflected a fall in total lymphocyte count. There was a progressive rise in lung lymph lymphocyte concentration and clearance (concentration × $\dot{Q}L$).

10^4 u/ml did not moderate TB-BSA passage through the EC barrier (Fig. 8). The addition of cytochalasin B, as a positive control, increased significantly ($p < 0.05$) TB-BSA passage across EC into the microcarrier matrix (Fig. 8).

Discussion

Interleukin-2 administration led to respiratory dysfunction manifest by pulmonary hypertension, hypoxemia, and increase in pulmonary microvascular permeability. These changes are associated with thromboxane generation, fall in circulating lymphocytes, lymphocytic infiltration of the lung, and appearance of these cells in lung lymph. IL-2 also induced a hyperdynamic state, characterized by increased cardiac output and fever.

The most striking effect of IL-2 was the altered microvascular permeability to protein evidenced by the three- to fivefold increase in $\dot{Q}L$ and rise in the L/P protein ratio.[6,19] The altered permeability did not require the infusion LAK cells, previously thought to be necessary.[3] The increase in permeability, coupled with the rise in MPAP, will further enhance transvascular filtration and contribute to the development of pulmonary edema.

The permeability increase, evident within 2 hours of the first administration of IL-2, was progressive over the course of the 5-day treatment period. Previous studies have reported that the "vascular leak syndrome" was noted only after 3 consecutive days of IL-2 administration.[3,4] Edema in these studies was estimated by measuring the wet to dry weight ratio or radiolabelled albumin uptake by tissue. Edema, an extreme consequence of increased permeability, might not have been detected earlier in those studies if the increased fluid filtered into the interstitium did not exceed the removal capacity of the lymphatics.[19] Only when this lymphatic capacity to clear the interstitium is exceeded does edema develop.[19] Furthermore, the data of previous studies are difficult to interpret since the methods used did not discriminate between the two common causes of tissue weight and albumin increase. Thus, a rise in wet/dry weight ratio or in radioactive albumin uptake could reflect an increase in intravascular volume due to vasodilitation. The sheep model with lung lymph fistula on the other hand, is a sensitive assay of micro-

TABLE 3. *Hematologic Changes Induced by II-2 or EXC*

	Pretreatment	Day 1 IL-2	Day 5 IL-2	Pretreatment	Day 1 EXC	Day 4 EXC
Blood WBC	8.4 ± 0.6	5.3 ± 0.9*	3.2 ± 1.2*	7.4 ± 0.2	5.6 ± 1.0	5.4 ± 0.5†
Granulocytes	3.5 ± 0.5	2.8 ± 0.4	1.4 ± 0.7*	3.6 ± 0.3	2.8 ± 0.2	2.6 ± 0.3
Lymphocytes	4.9 ± 1.1	2.4 ± 1.8*	1.1 ± 0.5*†	3.7 ± 0.5	2.7 ± 0.4	2.8 ± 0.4†
Monocytes	210 ± 7.0	384 ± 91	660 ± 95*§	160 ± 35	145 ± 39†	116 ± 46†
Eosinophils	0.8 ± 0.4	49 ± 7*	453 ± 84*‖	1.0 ± 0.5	1.4 ± 0.5†	1.1 ± 0.3†
Lung lymph lymphocytes	40.3 ± 3.9	41 ± 2	67.6 ± 8.0*	43.5 ± 5.0	15 ± 6*†	21 ± 8†
Lymph lymphocytes clearance × 10^3/30 minutes	89 ± 11	104 ± 50	450 ± 60*	110 ± 24	105 ± 24	82 ± 22†
Platelet	239 ± 36	147 ± 29*	42 ± 19*	306 ± 41	289 ± 34†	266 ± 39†

Blood and lymph cell counts are × 10^3/mm³, except monocytes and eosinophils, which are /mm³.

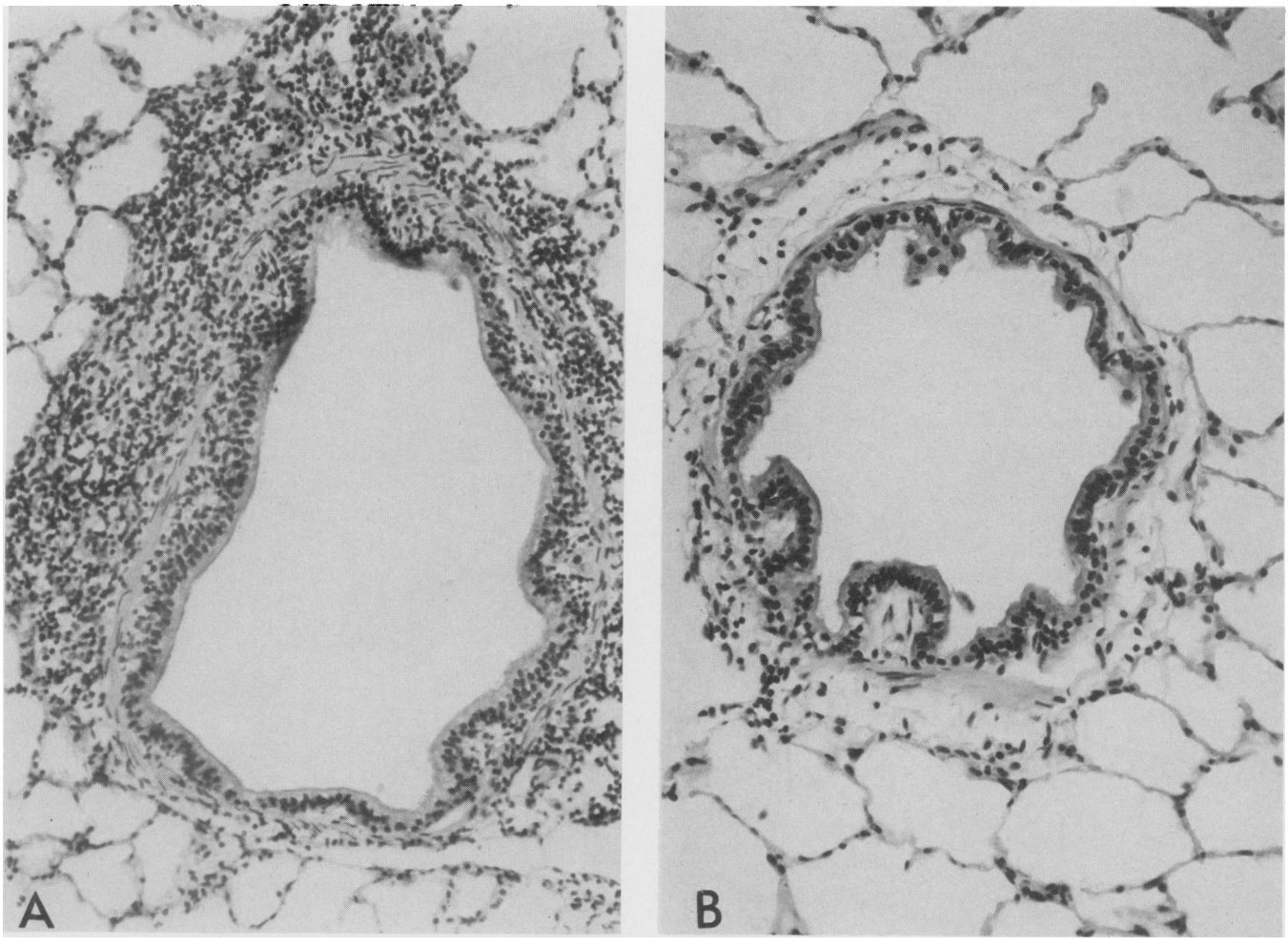
* $p < 0.05$ *vs.* pretreatment values.

† $p < 0.05$ between groups.

‡ Day 5 pretreatment value of 1.9 ± 0.3.

§ Pretreatment value of 462 ± 60.

‖ Pretreatment value of 390 ± 75, all significantly ($p < 0.05$) different from pretreatment values on Day 1.



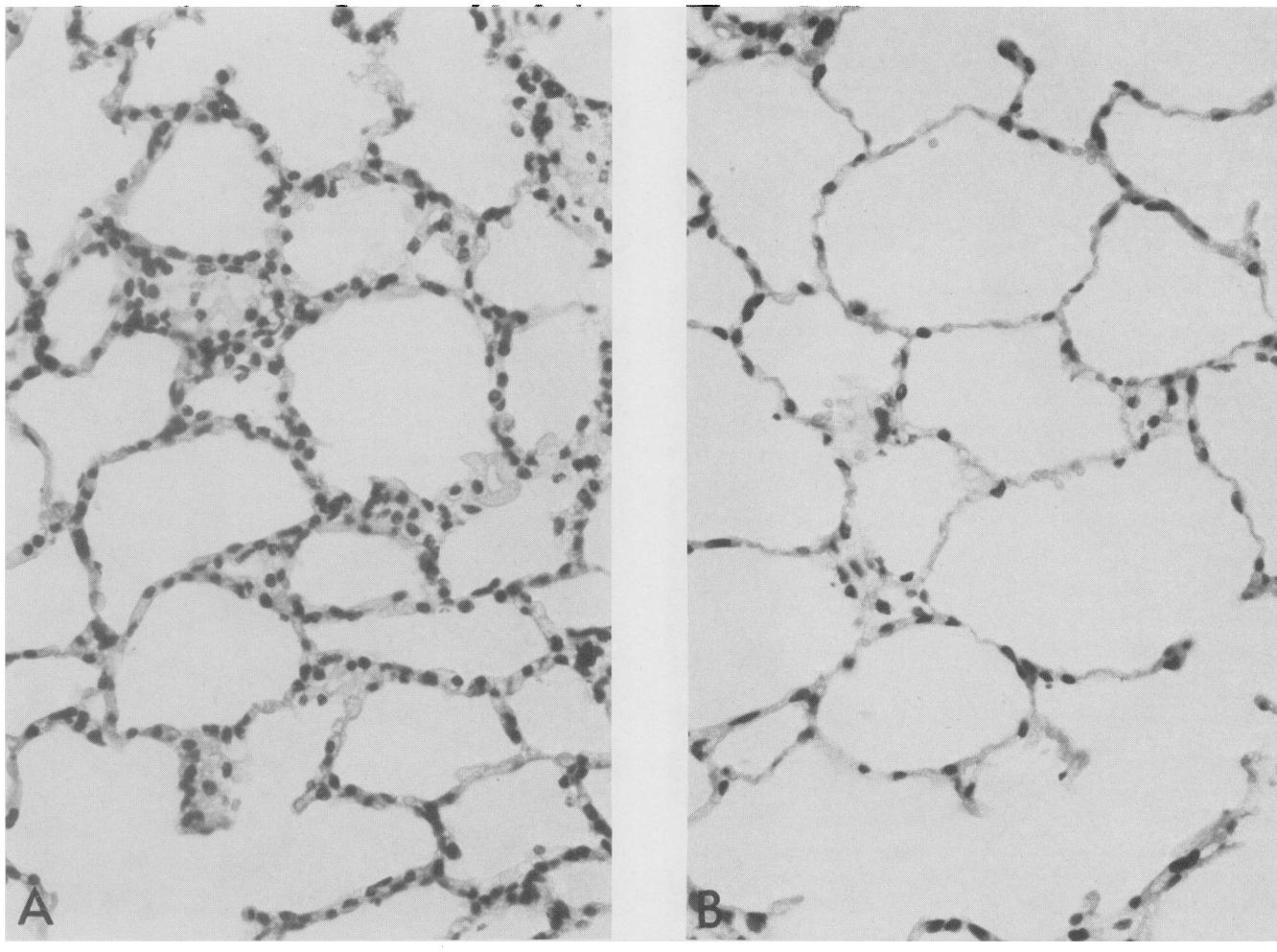
FIGS. 6 A and B. (A) Marked lymphocytic infiltration occurred around small airways after 5 days of IL-2 treatment (left panel). (B) Airways from EXC-treated animals appeared normal (right panel) (magnification × 600).

vascular permeability, allowing the early detection of permeability to protein.[6,19]

One possible limitation of the sheep lung lymph preparation is that the lymph collected from a postnodal lymphatic might reflect IL-2 induced permeability alterations of the lymph node. This is unlikely, since even after extensive changes in lymph nodes induced by irradiation, fluid transport through the associate lymphatics was not affected.[20]

In most other models of experimental lung injury such as endotoxemia or activated complement infusion, pulmonary hypertension and hypoxemia occur within a few minutes.[21,22] With IL-2, pulmonary dysfunction is delayed, appearing an average of 556 minutes after infusion. This suggests that IL-2 acts indirectly via other mediators such as lymphotcytes, a thesis consistent with the *in vitro* failure of IL-2 when incubated with EC to provoke a decrease in barrier function. Other observations lend support to the indirect action of IL-2 on permeability that is the absence of effect on EC cytoskeleton or morphology, parameters closely related to the size of interendothelial cell junctions.[23]

We speculate that IL-2 may exert its permeability effect through the action of stimulated white blood cells. Leukocytes are reported to be the effector cells in other models of lung injury such, as following complement activation, endotoxemia, microembolization, and limb ischemia.[24-27] However, in these settings neutrophils are the important mediators, whereas lymphocytes appear central after IL-2. The latter is suggested by the rapid clearance of lymphocytes from the peripheral circulation after IL-2 injection and their subsequent sequestration in the lungs. The marked lymphocytic accumulation in the lung may in part be due to proliferation of resident lymphocytes under the stimulation of IL-2.[28] It is also likely that IL-2 induces lymphocyte margination, transvascular diapedesis, and entrapment in the lungs as well as in other organs. The increase in the number of lymphocytes in lung lumph



FIGS. 7 A and B. (A) Diffuse lymphocyte sequestration in alveolar capillary beds was noted after 5 days of IL-2 administration (left panel). (B) Lung sections from EXC-treated sheep appeared normal (right panel) (magnification × 800).

may represent clearance of lymphocytes from the lung interstitium. It is also possible that this reflects a direct IL-2 effect on the caudal mediastinal lymph node.

In further support of the central role of lymphocytes in mediating permeability are the observations that IL-2, in a dose-dependent manner, leads to selective lymphocyte EC adherence.[29] Further, IL-2 activated lymphocytes are highly cytotoxic, not only to tumor cells, but also to EC.[29] Finally, that IL-2 induced permeability is a lymphocyte-mediated event is supported by the observation that IL-2 does not induce the "vascular leak syndrome" in nude mice or in mice immunodepressed with cyclophosphamide or total body in radiation, measures that eliminate the IL-2 induced proliferation of endogenous lymphocytes.[3,4]

Thromboxane (Tx)A_2, a vasoactive and vasotoxic agent, has been shown to be released after IL-2 administration, although the source of Tx synthesis is unclear. The observation that thrombocytopenia developed during IL-2 administration may indicate platelet activation and synthesis of Tx. However, platelets are not a significant source of Tx in the sheep,[30] therefore the IL-2 induced thrombocytopenia may reflect platelet aggregation secondary to Tx generation from other sources. Circulating neutrophils and activated lymphocytes can also synthesize TxA_2 in high concentrations and are more likely to be the synthetic source.[30,31] The high TxB_2 concentration in lung lymph does not resolve the relative role of lung parenchymal Tx synthesis and secretion into lymph versus Tx synthesis by lymphocytes contained in lymph.

Several properties of TxA_2 may be directly operative in inducing pulmonary dysfunction.[22,30] The smooth muscle constriction induced by TxA_2 can account for bronchospasm with resultant hypoxemia as well as for the pulmonary vasoconstriction and the pulmonary hypertension. Increased pulmonary microvascular permeability

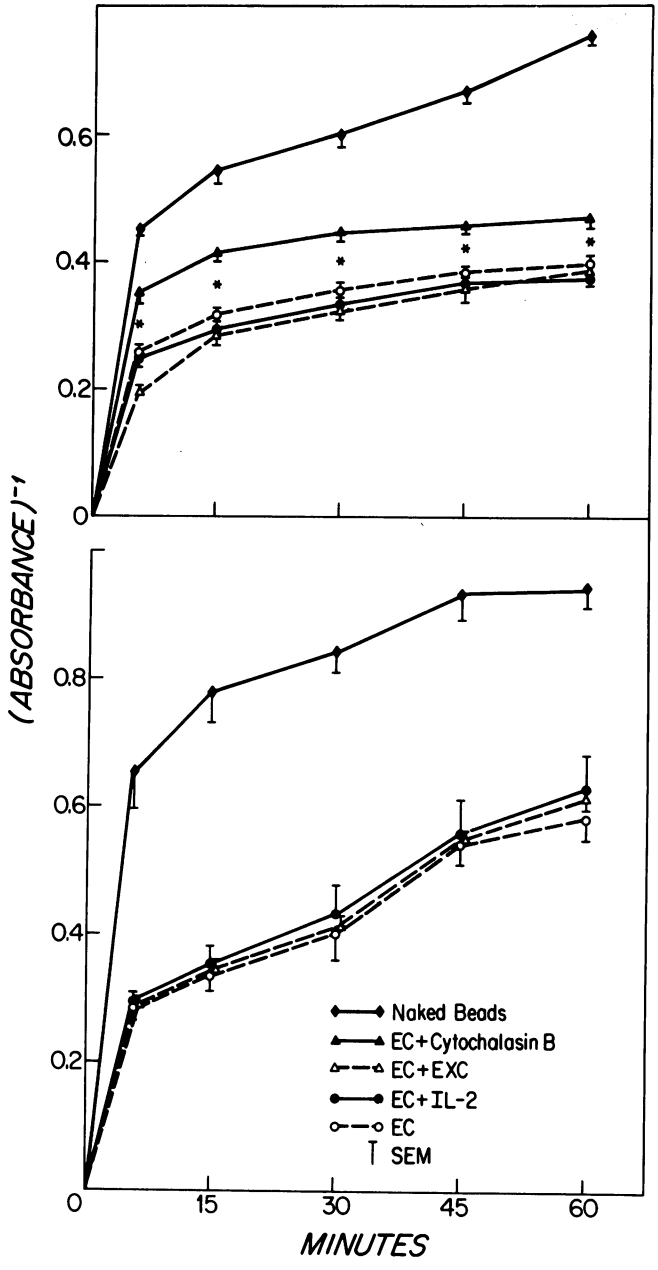


FIG. 8. IL-2, 10^4 U/ml or its EXC control did not alter barrier function after 1-hour (upper panel) and 4-hour (lower panel) incubations of EC-covered microcarrier beads. One-hour incubation with cytocholasin B induced permeability changes. Naked beads were highly permeable. Asterisks in the upper panel indicate $p < 0.05$ between cylocholasian B treated and untreated EC.

may also be mediated by TxA_2.[22,27,32] One mode of TxA_2 action in altering permeability is the disassembly of EC stress fibers.[23,33] This change in the endothelial cell cytoskeleton could modify interendothelial junctions and lead to reduced effectiveness of the microvascular barrier.[23,33] TxA_2 also enhances WBC diapedesis.[34] It may therefore mediate the lymphocyte sequestration that follows IL-2 administration. Other mediators of potential importance in mediating the pulmonary dysfunction and altered permeability are the leukotrienes, interleukin-1, interferon, and oxygen-free radicals.[2,4,35,36] IL-2 may also stimulate the complement system. This role is suggested by the similarity of the lung lymphosequestration seen after IL-2 and after prolonged and repeated complement activation.[37]

Many of the side effects encountered during IL-2 administration such as fever, chills, respiratory failure and increased permeability suggest the action of endotoxin, a consideration made possible since the IL-2 used is derived from *E. coli*.[38] Nevertheless, endotoxin is an unlikely cause of the observed changes after IL-2 therapy. The endotoxin concentration in the IL-2 preparation administered was less than 0.06 ng/kg. A dose of lug/kg was needed for a similar pulmonary response in sheep.[39] Further, there are basic differences between responses to IL-2 and endotoxin in the sheep.[39] In contrast to IL-2, the first two to three endotoxin doses lead to: immediate rather than delayed pulmonary hypertension, granulocytopenia rather than lymphopenia, and decreased cardiac output instead of the increase seen with IL-2. Further, repeated IL-2 infusions lead to progressive respiratory dysfunction, enhanced microvascular permeability, and thromboxane generation. By contrast, repeated doses of endotoxin lead to tachyphylaxis and attenuation of the respiratory dysfunction.[39] Finally, endotoxin causes direct damage to endothelial cells with cell retraction and pyknosis,[40] whereas EC morphology is unaffected by IL-2.

In summary, infusion of IL-2 leads to cardio-pulmonary alterations within 2 hours. There is pulmonary lymphosequestration, increased permeability, and progressive pulmonary dysfunction. The pulmonary injury is not due to a direct effect of IL-2 on the lung microvasculature.

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