Role of Thromboxane in Interleukin 2-induced Lung Injury in Sheep

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ABSTRACT

Interleukin (IL)-2 administration leads to respiratory dysfunction due to increased vascular permeability. This study examines the role of thromboxane (TXA2) in IL-2 induced lung injury in sheep with chronic lung fistulae. This preparation enables evaluation of permeability prior to the development of gross edema. IL-2, 10^9 units/kg (n = 6), or its excipient control (n = 5) was given as an i.v. bolus over 2 min. After 2 h of IL-2 administration, plasma TXB2 increased from 168 to 385 pg/ml (P < 0.05) and lung lymph TXB2 from 235 to 694 pg/ml (P < 0.05). Mean pulmonary artery pressure (NIPAP) rose from 13 to 29 mm of Hg (P < 0.05) at 30 min and remained elevated for 4 h while the pulmonary artery wedge pressure was unchanged at 4 mm of Hg. Arterial oxygen tension (Pao2) fell from 88 to 77 mm of Hg (P < 0.05). Lung lymph flow (QL) rose from 2.1 to 3.8 ml/30 min (P < 0.05) at 1 h and to 6.4 ml/30 min at 3 h. This rise coincided with an increase in the lymph/plasma (L/P) protein ratio from 0.67 to 0.77 (P < 0.05). In contrast, the non-IL-2-infused sheep (n = 3) recruitment of the lung vasculature by left atrial balloon inflation led to a rise in QL from 2.4 to 8.2 ml/30 min, whereas the L/P ratio declined from 0.62 to 0.25, suggesting that the protein-rich lymph flow after IL-2 administration reflected increased microvascular permeability. In further proof of an increase in permeability, IL-2 administration into sheep (n = 2) with an inflated left atrial balloon led, after a pressure-independent L/P protein ratio had been achieved, to an increase in L/P protein ratio and decrease in protein reflection coefficient. At 2 h after IL-2, the blood leukocyte count fell from 8156 to 4375/mm^3 (P < 0.05) primarily due to a 73% drop in lymphocytes. The platelet count declined from 292 to 184 x 10^6/mm^3 (P < 0.05). Body temperature fell from 38.9-40.3°C (P < 0.05), and shaking chills were common. Pretreatment with the Tx synthetase inhibitor OKY 046 (n = 7) lowered baseline plasma and lymph TXB2 levels to 22 and 52 pg/ml (P < 0.05) and prevented the IL-2-induced increase in plasma and lymph TXB2 levels (P < 0.05). Tx inhibition prevented the increase in MPAAP at 30 min and limited its rise at 2 to 14 mm of Hg, a value lower than untreated IL-2-infused animals (P < 0.05). Pao2 decreased from 89 to 81 mm of Hg (P < 0.05). The initial increase in QL and the L/P protein ratio were delayed by 1.5 h. Thereafter, there was a lesser permeability effect, since QL but not the L/P ratio reached levels similar to IL-2-treated controls. The leukopenia and lymphopenia were unaffected by Tx inhibition, while the fall in platelet count was prevented (P < 0.05). The rise in temperature to 39.4°C was lower than IL-2 controls (P < 0.05). Shaking chills were not seen. Infusion of the excipient control did not affect plasma or lymph TXB2 levels, but there were increases in MPAAP from 12 to 16 mm of Hg (P < 0.05) and QL from 2.5 to 3.7 ml/30 min, while the L/P protein ratio fell from 0.71 to 0.63 (P < 0.05). Body temperature rose from 39.2-39.8°C. The Pao2, WBC, lymphocyte, and platelet counts were unaffected. Incubation of IL-2 with sheep neutrophils but not sheep lymphocytes, platelets, or bovine pulmonary artery endothelial cell monolayers led to TXB2 generation. These data indicate that IL-2 leads to pulmonary hypertension and increased microvascular permeability. The former effect is mediated by TXA2, derived in part from neutrophils. The role of Tx in mediating IL-2-induced permeability is less clear. It can be due to a direct action on the vascular barrier or secondary to induction of pulmonary hypertension and increased filtration pressure.

INTRODUCTION

The major toxicity of IL-2 therapy for malignant disease is increase in microvascular permeability resulting in tissue edema and pulmonary dysfunction (1, 2). The IL-2-induced lung injury may lead to respiratory failure and result in cessation of treatment. In a previous study, we have shown that the vascular barrier is not directly affected by IL-2 (3). Thus, IL-2 when incubated with an EC monolayer does not provoke a decrease in barrier function. Further, IL-2 fails to induce the "vascular leak syndrome" in nude mice or in mice immunodepressed with cyclophosphamide or total-body radiation (2). These observations indicate that secondary mediators are involved.

One of the consequences of IL-2 administration is the synthesis and release of TXA2 (3). This vasotoxic and proaggregatory prostanoit has been implicated in the respiratory failure accompanying endotoxemia, complement activation, aspiration, microembolization, and ischemia-reperfusion of the lower torso (4-7). The present study was designed to evaluate the cellular source of Tx and to test its role in IL-2-induced lung injury.

MATERIALS AND METHODS

Sheep Preparation. Yearling female interbred sheep (n = 23) weighing 22 to 40 kg were prepared with chronic lung lymph fistulae according to a modification of the technique described by Staub (3, 8). Briefly, through a right thoracotomy the efferent duct of the caudal mediastinal lymph node was cannulated, the distal portion of the lymph node below the level of the inferior pulmonary ligament was ligated, and the diaphragm around the lymph node was circumferentially cauterized. All visible systemic lymph tributaries to its proximal portion were cauterized or ligated to minimize extrapulmonary contamination of collected lymph (9). A thermistor-tipped pulmonary arterial catheter (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 to 5 days when animals appeared vigorous, were afebrile, and had a steady flow of blood-free lymph, the experiment was conducted. In three sheep, 5 days after lung lymph cannulation, an anterolateral left thoracotomy was performed. A 16-Fr silicone elastomer-coated Foley catheter with a 30-ml inflatable balloon (No. S616; American Pharmacal, Valencia, CA) was inserted into the left atrium. These sheep were allowed 5 more days of recovery before the experiments.

Cardiopulmonary Function. Stain-gauge transducers (Model D-240; Bently Laboratories, Inc., Irvine, CA) were used to measure the following pressures: MAP; MPAAP; PAWP; and LAP. The Pmv was calculated from the Gaar equation, Pmv = PAWP + 0.4 (MPAP - PAWP) (10).

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3 The abbreviations used are: IL-2, interleukin 2; EC, endothelial cell; Tx, thromboxane; MAP, mean arterial pressure; MPAAP, mean pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; LAP, left atrial pressure; Pmv, pulmonary microvascular pressure; PG, prostaglandin; L/P, lymph/plasma; QL, lung lymph flow; ed, osmotic reflection coefficient; EXC, excipient control; PAEC, pulmonary artery endothelial cell; HBSS, Hank’s balanced salt solution; DMEM, Dulbecco’s modified Eagle’s medium; TNF, tumor necrosis factor; Pao2, arterial oxygen tension.

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Output was measured in triplicate by thermodilution (Model 5IL-2; 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 (Models 813 and 282; Instrumentation Laboratory, Lexington, MA).

Hematology. Circulating platelets and WBC were counted by means of phase microscopy. Differential counts were made on Wright's stained blood smears.

Biochemical Assays. Plasma and lymph concentrations of TxB₂ and 6-keto-PGF₁α, the stable hydrolysis products of TxA₂ and prostacyclin, were measured in duplicate by radioimmunoassay (11). Blood was drawn into cooled syringes containing 0.5 ml of 0.07 M EDTA and 0.09 M aspirin. The blood was immediately centrifuged at 1500 x g at 4°C for 20 min, and the plasma was separated and stored at —20°C until assayed.

Lung lymph was collected at 30-min intervals in cold graduated tubes containing 0.07 M EDTA and 0.09 M aspirin adjusted to 5% volume of collected lymph. The lymph was then centrifuged at 1500 x g at 4°C for 20 min, and the supernatant was separated and stored at —20°C until assayed for TxB₂ and 6-keto-PGF₁α. Lymph and plasma total protein concentrations were determined in duplicate by the spectrophotometric protein dye method described by Bradford (13). The L/P protein ratio was calculated and multiplied by QL to obtain the lymph protein clearance. The χ₀ for total protein was calculated using the minimum L/P protein ratio achieved at a steady state during bolus infusion when LAP is increased and QL is high. At this point the L/P protein ratio becomes independent of the filtration rate and approaches (1 - χ₀) (10, 14).

IL-2. The recombinant interleukin-2 (ala-125) and its 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 (15). After purification, the material had a specific activity of 2.8 x 10⁵ units/mg of protein. IL-2 activity was measured in a standard assay using the CTLL-2-dependent cell line (16). 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 mM). No protein was contained in the EXC control. Final container testing was carried out by 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 repositioned as necessary. Baseline measurements were obtained for 2 h. IL-2 (10¹⁰ units/kg) or its EXC control (n = 5) was then administered as a bolus infusion over 2 min through the central venous line. Sheep receiving IL-2 were pretreated with either i.v. saline (n = 6) or the Tx synthetase inhibitor OKY 046 (5 mg/kg) given as a bolus injection over 1 min, starting 30 min prior to IL-2 administration and repeated every 2 h (n = 7) (4). Cardiopulmonary function was continuously monitored while blood and lymph were sampled every 30 min for 8 h following IL-2 or EXC administration.

In non-IL-2-infused control sheep (n = 3) after a baseline period of 1 h, the LAP was increased for 3 h to 18 to 20 mm of Hg by inflation of the Foley balloon catheter. The purpose of balloon inflation with the resultant increase in LAP, MPAP, and pulmonary microvascular surface area was to raise QL and contrast the resultant changes in L/P protein ratio with those noted after IL-2 administration (10, 17). In two additional animals, IL-2 was administered 2.5 h after left atrial balloon inflation, when QL had stabilized at high flow and the L/P protein ratio was independent of pressure (14, 17). The left atrial balloon was kept inflated for 3 additional h. Plasma samples were taken every 30 min, and lymph was collected every 15 min in these sheep.

Animals used in this study were maintained in accordance with the guidelines of the Committee on 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 78-23, revised 1978).

Cell Incubation with IL-2. In order to identify the cellular source of Tx in response to IL-2 administration, in vitro studies were conducted with sheep platelets, granulocytes, or lymphocytes as well as bovine PAEC monolayers. The latter were selected as being more characterized and defined, compared to ovine PAEC which are rarely studied. Blood was drawn from healthy sheep via jugular venipuncture. Immediately upon collection, the whole blood was mixed (9:1, v:v) with 0.1 M EDTA 4°C for 15 min. Theuffy coat was aspirated. Contaminating erythrocytes were removed by hemolysis, with 2 volumes of distilled water at 4°C for 30 s. The osmolality was restored by the addition of a double concentration of HBSS (Sigma Chemical Co., St. Louis, MO). A leukocyte pellet was obtained by centrifugation, and the cells were suspended in 1.0 ml of HBSS. Neutrophils and lymphocytes were separated as described by Cooper (18). Briefly, aliquots of the leukocyte suspension were layered over Percoll (Sigma) density gradients. Following centrifugation at 900 x g for 15 min, the neutrophil band was aspirated. The neutrophils were washed with HBSS to remove the Percoll and then resuspended in 1.0 ml of HBSS. Upon distribution of leukocytes across the Percoll gradients, the lymphocyte band and the surrounding bands were aspirated and then centrifuged at 260 x g, 4°C for 10 min. The isolated populations from the Percoll gradients yielded neutrophils with a purity of 83 ± 3%. The contaminant was 17 ± 3% lymphocytes. The lymphocyte purity was 89 ± 2% with 11 ± 2% neutrophils. Three preparations were evaluated for each cell type. Because of platelet contamination (30%) present in the first experiment that may have contributed to Tx generation, the following two studies were performed. Sheep were depleted of platelets by treatment over 2 days with repeated i.v. injections of rabbit antiplatelet platelet serum (19). The resultant platelet count was <100/mm³. No platelet contamination was found following WBC isolation from blood obtained from platelet-depleted sheep.

Viability of the separated neutrophils and lymphocytes was tested by measuring the differential uptake of ethidium bromide and fluorescein diacetate (20) and was 98% for neutrophils and 78% for lymphocytes.

Centrifugation of whole blood treated with EDTA at 260 x g for 5 min in siliconized tubes was used to separate platelet-rich plasma. WBC were counted using a Coulter Counter (ZBI; Coulter Electronics, Hialeah, FL) and platelets by phase microscopy. The separated cells were suspended in HBSS to obtain a final concentration of 2 x 10⁶ cells/ml. They were incubated with IL-2 at concentrations of 10¹⁰ to 10¹³ units for 4 h at 37°C in a shaking bath. At the end of the incubation period, aspirin (50 µg/ml) was added to stop further Tx generation. The suspensions were immediately centrifuged at 1500 x g at 4°C for 20 min, and the supernatant was separated and stored at —20°C until assayed for TxB₂.

Some PAECs were obtained as previously described (21). ECs were identified by their characteristic growth morphology, specific low density lipoprotein uptake, and presence of Factor VIII antigen (22, 23). Primary cultures were subcultured a maximum of 3 times before seeding onto a 24-well Linbro tray in DMEM (Gibco, Grand Island, NJ) supplemented with 10% fetal calf serum (Hicon Lab., Inc., Logan, UT) and 1% antibiotic and antimycotic solution (Sigma). At 4 days postconfluence, the cell monolayers were washed with DMEM and exposed to IL-2 in DMEM at concentrations of 10¹ to 10² units for 4 h. The supernatant was then removed and immediately frozen at —20°C until assayed for TxB₂. The cells in each well were trypsinized and counted using a Coulter Counter.

All data are presented in the text and figures as mean ± standard error. Statistics are based on analysis of variance, paired and nonpaired t tests. When multiple comparisons were done, the Bonferroni procedure was applied (23). Significance was accepted if P < 0.05.

RESULTS

Interleukin 2. IL-2 administration produced an increase in plasma TxB₂ levels from 168 ± 30 to 265 ± 50 pg/ml at 30 min and to a maximum of 388 ± 111 pg/ml at 2 h (P < 0.05) (Fig. 1). TxB₂ levels then declined and at 4 h returned to base line. concentration in lung lymph rose from 235 ± 28 to 694 ± 123 pg/ml at 2 h (P < 0.05), values higher than plasma (P < 0.05) (Fig. 1). Plasma and lung lymph 6-keto-PGF₁α levels were...
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Fig. 1. IL-2 administration led to an increase in plasma TxB2 levels. A higher and more sustained rise of TxB2 concentration occurred in lung lymph. OKY 046 decreased base-line plasma and lymph TxB2 levels and prevented IL-2-induced Tx synthesis. EXC control infusion did not alter TxB2 concentration in plasma or lymph. *, significance relative to base-line values; †, significant differences between saline and OKY 046 treatments; ‡, significant differences between IL-2 and EXC controls. Bars, SE.

Fig. 2. IL-2 led to a rise in MPAP that peaked at 1 h and thereafter remained elevated for 4 h. Thromboxane inhibition by OKY 046 prevented the early increase in MPAP and limited its later rise. EXC control caused a mild, short-lived rise in MPAP. *, significance relative to base-line values; †, significant differences between saline and OKY 046 treatments; ‡, significant differences between IL-2 and EXC controls.

Fig. 3. Following IL-2 administration, QL increased immediately along with a rise in the L/P protein ratio resulting in a significant increase in lymph protein clearance. Pretreatment with OKY 046 prevented the initial increases in QL, L/P, and lymph protein clearance and limited the late rise in the L/P protein ratio. EXC control led to small increases in QL and lymph protein clearance along with a decline in the L/P protein ratio. *, significance relative to base-line values; †, significant differences between saline and OKY 046 treatments; ‡, significant differences between IL-2 and EXC controls.

13 ± 2 mm of Hg (P < 0.05), while the PAWP was unchanged from base-line levels of 4 ± 1 mm of Hg. The PaO2 decreased from 88 ± 3 at base line to 77 ± 3 mm of Hg at 3 h (P < 0.05) and thereafter returned toward pretreatment levels. The PaCO2 was unchanged from 34 ± 4 mm of Hg.

IL-2 led to a rapid increase in QL from 2.2 ± 0.3 to 3.1 ± 0.3 ml/30 min at 30 min (P < 0.05) (Fig. 3). The QL then steadily increased, reaching a maximum of 6.4 ± 1.6 ml/30 min at 3.5 h. Thereafter QL slowly decreased, reaching base-line levels at 8 h. The L/P protein ratio rose from 0.67 ± 0.03 to 0.71 ± 0.03 at 30 min and peaked at 0.77 ± 0.05 (P < 0.05), 3.5 h post-IL-2 administration. This coincided with the maximal QL (Fig. 3). The lymph protein clearance increased 3-fold from 1.5 ± 0.2 to 4.4 ± 1.2 ml/30 min (P < 0.05) between 3

unchanged from base-line values of 46 ± 13 and 75 ± 21 pg/ml, respectively.

IL-2 led to a rise in MPAP from 13 ± 1 to 21 ± 4 mm of Hg at 30 min (P < 0.05) (Fig. 2). At 1 h MPAP peaked at 23 ± 4 mm of Hg and thereafter gradually declined, reaching base-line values after 4 h (Fig. 2). There was a rise in Pmv from 8 ± 1 to
and 4 h post-IL-2 and then gradually returned to pretreatment values (Fig. 3).

Following IL-2 administration, there were a directional increase in the CO from 5.4 ± 0.4 to 6.6 ± 1.1 liters/min and a decrease in MAP from 88 ± 4 to 81 ± 5 mm of Hg, but neither change was significant. Core temperature rose from 38.9 ± 0.3 to 40.3 ± 0.4°C (P < 0.05), and shaking chills occurred in all animals.

IL-2 led to a fall in platelet count from 292 ± 16 to 184 ± 51 x 10³/mm³ (P < 0.05) (Fig. 4). Circulating WBC also declined from 8156 ± 831 to 4375 ± 777/mm³ (P < 0.05) (Fig. 4), mainly reflecting a fall in the number of lymphocytes from 61 ± 13% to 20 ± 17% of total WBC (P < 0.05). Platelet and WBC counts returned to base-line values at 8 h.

Increased Left Atrial Pressure. This led to increases in LAP from 4 ± 2 to 19 ± 2 mm of Hg and MPAP from 12 ± 2 to 22 ± 3 mm of Hg. During the 3 h of balloon inflation, QL rose from base-line levels of 2.4 ± 0.4 to 8.2 ± 1.2 ml/30 min. In contrast to the IL-2-infused group, where the high QL coincided with a rise in the L/P protein ratio, here the ratio declined from 0.62 ± 0.03 to 0.25 ± 0.02 (Fig. 5). Further, IL-2 infusion after 2.5 h of balloon inflation when a pressure-independent L/P protein ratio had been achieved led to an additional rise in QL which was accompanied by an increase in the L/P protein ratio and a decrease in the calculated protein reflection coefficient (rd) (Table 1; Fig. 6).

Interleukin 2 + OKY 046. Pretreatment with the Tx synthetase inhibitor lowered base-line plasma and lymph TxB₂ levels to 22 ± 16 and 52 ± 22 pg/ml, respectively (P < 0.05), and prevented the IL-2-induced increase in TxB₂ (P < 0.05) (Fig. 1). The increase in MPAP was largely prevented. Only after 2 h was a slight rise to 14 ± 1 mm of Hg noted (P < 0.05), a value lower than in IL-2-treated animals (P < 0.05) (Fig. 2). The Pmv rose insignificantly from 7 ± 1 to 9 ± 1 mm of Hg. Inhibition of Tx did not prevent the decrease in PaO₂ from 89 ± 5 to 81 ± 2 mm of Hg (P < 0.05). The PaCO₂ was unchanged from 32 ± 4 mm of Hg.

Inhibition of Tx synthesis led to a decline in the L/P protein ratio from 0.71 ± 0.02 to 0.66 ± 0.02 (P < 0.05) (Fig. 5). For 1.5 h after IL-2 administration, OKY 046 prevented the IL-2-induced rise in QL and lymph protein clearance (P < 0.05) (Fig. 3). Thereafter, both QL and lymph protein clearance increased, similar to IL-2-treated animals. In contrast to IL-2 treatment alone, where the L/P protein ratio rose immediately and was significantly above base-line values between 2.5 and 5 h, therapy with OKY 046 tended to maintain an L/P ratio.
lower than pretreatment values for the entire period of monitoring except once, at 3 h (Fig. 3).

The CO and MAP were unchanged from base-line levels of 5.7 ± 0.5 liters/min and 86 ± 4 mm of Hg. The temperature rose from 38.8 ± 0.1 to 39.4 ± 0.2°C (P < 0.05), lower than IL-2-treated sheep (P < 0.05).

The IL-2-induced leukopenia and lymphopenia were unaffected by Tx inhibition (Fig. 4). WBC count decreased from 8896 ± 778 to 4792 ± 840/mm³, and the lymphocytes declined from 58 ± 9 to 24 ± 19% (P < 0.05) similar to the IL-2 group. The reduction in platelet numbers from the base-line value of 336 ± 128 × 10³ (Fig. 4) was prevented (P < 0.05).

Excipient Controls. In contrast to IL-2 treatment, EXC administration did not cause increases in plasma or lung lymph TxB₂ levels (P < 0.05). Both remained unchanged from base-line values of 215 ± 38 and 280 ± 91 pg/ml, respectively (Fig. 1). EXC infusion led to a rise in MPAP from 12 ± 1 to 16 ± 2 mm of Hg (P < 0.05) at 30 min, a value lower than in IL-2-treated animals (P < 0.05) (Fig. 2). The Pmv rose from 8 ± 1 to 11 ± 2 mm of Hg (P < 0.05). The PaO₂ remained unchanged from 89 ± 3 mm of Hg, a value higher than in IL-2-treated sheep (P < 0.05). The QL increased from 2.5 ± 0.3 to 3.7 ± 2.8 ml/30 min (P < 0.05) (Fig. 3), but this was accompanied by a decline in the L/P protein ratio from 0.71 ± 0.03 to 0.63 ± 0.03 (P < 0.05). The increases in QL and lymph protein clearance were both lower than in IL-2-treated sheep (P < 0.05) (Fig. 3). EXC control did not affect CO or MAP. The temperature rose from 39.2–39.8°C (P < 0.05), lower than in the IL-2 group (P < 0.05). The WBC count was unchanged from base-line values of 8650 ± 610/mm³ (Fig. 4) with 55 ± 12% lymphocytes. Also unaffected was the platelet count that remained at 309 ± 49 × 10³/mm³ (Fig. 4).

Cellular Source of Tx. In vitro studies of IL-2 incubation with various cell types showed that TxB₂ was generated from isolated neutrophils in a dose-dependent manner (18 ± 6 to 71 ± 3 pg/10⁶ cells with 10 units of IL-2 and to 107 ± 65 pg/10⁶ cells with 10³ units of IL-2). In contrast, platelets (7 ± 1 pg/10⁶ platelets), lymphocytes (29 ± 19 pg/10⁶ cells), and pulmonary arterial endothelial cells (184 ± 22 pg/10⁶ cells) were not stimulated by IL-2 to synthesize TxB₂ (Fig. 7).

### DISCUSSION

Interleukin 2 administration leads to respiratory dysfunction manifested by pulmonary hypertension, decline in PaO₂, and increased lung microvascular permeability to protein. These changes are associated with Tx generation and a fall in circulating lymphocytes.

Previous studies have shown lesser increases in MPAP following IL-2 administration in sheep (2, 4, 24). However, hemodynamics in these sheep were monitored only at 24-h intervals, and the maximal changes could have been missed, given the observation that the rise in MPAP is transient and only lasts 1 to 4 h after a bolus injection. Further, the mode of IL-2 administration may have influenced the magnitude and time course of development of hemodynamic alterations. Thus, in the present study the rapid bolus injection may result in higher IL-2 blood levels, relative to those resulting from IL-2 infusion over 30 min or 72 h (24, 25).

The increase in lung permeability is indicated by the 3-fold rise in QL along with a high L/P protein ratio resulting in protein-rich lymph flow. These changes occurred 3 h after IL-2 administration when MPAP and Pmv almost returned to normal. Thus, these lymph data are unlikely due to increases in capillary surface area or filtration pressure. Further, changes in surface area or pressure are associated with an increase in QL and by an unchanged or reduced L/P protein ratio. This is a consequence of the movement of more water than protein through a normal microvascular barrier into lymph (8, 14, 17).

Such responses were demonstrated by inflating a left atrial balloon in non-IL-2-infused control sheep (Fig. 5). On the other hand, IL-2 infusion after a pressure-independent L/P protein ratio had been achieved, in the setting of increased left atrial pressure, led to rises in QL and the L/P protein ratio as well as a fall in the protein reflection coefficient, documenting increased vascular permeability (8, 14, 17). Finally, that the IL-2-induced changes in lymph indicate increased microvascular permeability and are not a consequence of pressure is also suggested by the observation that increases in QL and L/P occurred, although delayed when the rise in MPAP was prevented by OKY 046. These alterations in the absence of forces that could increase surface area or filtration pressure argue strongly for increased permeability. Nevertheless, the early phase, 1.5 h after IL-2 administration, of increased permeability is not proven, since during that period there were only mild increases in QL and the L/P protein ratio, changes that could be explained by increased filtration pressure secondary to the pulmonary hypertension.

Increased permeability is evident within 2 h following IL-2 administration. In reports by others, permeability manifest by edema formation was detected after 3 or more days of IL-2 treatment (2, 26). In these studies edema was assessed by changes in the wet/dry weight ratio of the lung or tissue uptake of radiolabeled albumin. These latter studies done without tagging RBC to dissociate between intravascular and extravascular albumin are a less sensitive assay of microvascular permeability than the lung lymph preparation. Thus, edema, the extreme consequence of increased permeability, cannot be detected until there is a marked imbalance between the increased fluid and protein filtered into the interstitium and the removal capacity of the lymphatics (17). In contrast, the monitoring of lymph flow and the L/P ratio yields early information regarding changes in microvascular permeability. In fact, increased wet/dry weight ratio was detected in sheep after 72 h of IL-2 treatment (24).
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Other studies (24, 25) using the lung lymph preparation have also suggested that IL-2 induces increased permeability. However, lymph data in these experiments were only obtained 72 h after initiation of IL-2 treatment, in acutely prepared, anesthe-

ized sheep where the surgical trauma itself may have altered vascular permeability (27). A recent study using the chronic lung lymph preparation in awake sheep treated with IL-2 suggests that the lymph data are compatible with a modest increase in vascular permeability but acknowledges the significant con-

tribution of the hemodynamic changes to the altered lymph dynamics (28).

Thromboxane A₂, a vasoactive and vasotoxic agent (4), has been shown to be released following IL-2 administration. Although platelets are known to be a rich source of Tx (4), the present in vitro data do not indicate that IL-2 stimulates platelet synthesis. Further, ruminant platelets have little capacity for Tx generation (29). Paradoxically, it is likely that Tx produced from sites other than platelets leads to platelet aggregation and loss from the circulation. The fact that OKY 046 prevents a decline in platelet numbers supports this thesis. Endothelium and lymphocytes are other potential sources of TxA₂ (4, 30), but our data do not indicate these sites to be the source of Tx after IL-2 administration. On the other hand, neutrophils are activated directly or indirectly by IL-2 to synthesize Tx. Since the neutrophil preparations contained 17% lymphocytes, it is possible that IL-2-activated lymphocytes stimulate the neutrophils to release Tx. Despite these considerations, neutrophils do not appear to be the major source of IL-2-induced Tx synthesis in vivo, since their depletion in sheep by hydroxyurea does not prevent the rise in Tx concentration in plasma and lung lymph following IL-2 administration nor does it amelio-
rate the pulmonary hypertension and increased permeability. The high and sustained TxB₂ concentration in lung lymph, higher than plasma, strongly suggests a pulmonary site of origin. Lung parenchyma and especially vascular and alveolar macrophages could be responsive to IL-2 stimulation (31). This is further suggested by the observation that, in isolated guinea pig lungs perfused with a cell-free solution, IL-2 caused an increase in pulmonary vascular resistance (32). In addition to these considerations, the use of IL-2 in cancer patients could lead to tumor stimulation and Tx synthesis (4).

Several properties of TxA₂ may be important in mediating IL-2-induced lung injury (4). The vasoconstrictive effects of Tx appear responsible, in large part, for the pulmonary hyperten-

sion. This is strongly supported by the observation that inhibition of TxA₂ synthesis attenuates the rise in MPAP. However, the peak in plasma Tx occurs at 2 h, whereas the maximal increase in MPAP occurs at 1 h. This temporal dissociation may be related to tachyphylaxis to a prolonged Tx stimulus. Thromboxane can also induce bronchoconstriction which could lead to regional decreases in the ventilation/perfusion ratio and a reduction in PaO₂. However, this putative bronchoconstrictive property of TxA₂ does not appear to be important in the IL-2-

induced lung injury, since OKY 046 did not maintain PaO₂.

The early changes in fluid dynamics possibly representing an increase in permeability after IL-2 may be mediated by TxA₂. This conclusion is based on the ability of OKY 046 to prevent the initial increases in QL, the L/P protein ratio, and lymph protein clearance (Fig. 8). These early changes in QL and the L/P protein ratio appear not to be due entirely to pulmonary hypertension which would have caused a rise in QL and no change or a fall in the L/P ratio. The prevention of the pul-

monary hypertension by OKY 046 was therefore not likely to be its major mechanism of action. However, an alternative explanation is also possible. The IL-2-induced pulmonary hyper-

pertension may magnify the impact of increased permeability by increasing filtration pressure. On the other hand, in OKY 046-treated animals, the pulmonary hypertension was pre-

vented, and therefore this amplification of the early lymph response was eliminated. Thus, it is plausible that the early increase in lung vascular permeability became overt in the group given IL-2 alone and was masked in the OKY 046-treated animals.

Thromboxane A₂ can directly moderate the microvascular barrier by altering EC architecture and cytoskeleton. The mechanism is by the disassembly of actin microfilaments (33). These elements appear to regulate EC motility, structural relationship to adjacent cells, and barrier function (33-35). Thromboxane leads to disassembly and disruption of cytoskeletal actin microfilaments (33, 35). This is associated with widening of interendothelial tight junctions and increased permeability to protein (35). Thromboxane can also affect microvascular permeability through its interaction with leukocytes. Thus, Tx enhances neutrophil as well as monocyte-endothelial interaction and adhesion and promotes leukocyte diapedesis through the endo-

thelial barrier (36, 37). These events may in turn lead to increased permeability if the white cells are activated. This same mechanism of Tx moderation of permeability may be one mechanism underlying tumor metastasis. Thus, the reduced metastatic rate and improved survival in rats bearing prostate adenocarcinoma and treated with a Tx blocker (4, 38) may be related to a "tightened" vascular barrier and inhibition of tumor cell-endothelial adhesion and diapedesis. Improved survival rate with Tx blockade may also be related to immunomodulation properties of Tx (4). Thus, Tx has the ability to depress cyto-

toxicity of activated lymphocytes and natural killer cells, an effect that is prevented by a Tx receptor antagonist (39).

The simple finding of an elevation in plasma Tx levels does not document its role in mediating increased permeability. Thus, in some experimental settings such as endotoxemia in which increased plasma Tx concentrations are found, other mediators of permeability are of paramount importance (40). In other settings such as lung injury induced by ischemia-

Fig. 8. IL-2 administration led after 90 min to an increase in lung lymph protein clearance (early phase) that peaked after 3 h (late phase). Thromboxane inhibition by OKY 046 prevented the early phase changes. *, significance relative to base-line values; †, significant differences between groups.

* Unpublished observations in two sheep.
ROLE OF TXA₂ IN IL-2-INDUCED LUNG INJURY IN SHEEP

reperfusion, microembolization, complement activation, or acid aspiration, Tx appears central in mediating microvascular permeability (4-7). That Tx can induce permeability is further documented by the observation that Tx-mimic administration in sheep led to a decrease in the protein reflection coefficient and, when applied to EC monolayers, led to a decrease in the barrier function (35). Further, Tx blockade enhances the barrier function in vitro and in vivo, suggesting a role of Tx in modulating the vascular barrier (35).

The observation that Tx inhibition did not affect the late increase in permeability indicates that it is not the only mediator of IL-2-induced injury. Other agents, such as oxygen-derived free radicals, endoperoxides, leukotrienes, the complement system, as well as additional cytokines such as interleukin 1 and γ-interferon, may play a role. In addition, IL-2 triggers the production and release of TNF (41). This inflammatory mediator leads to pulmonary sequelae and an increase in vascular permeability similar to IL-2 (42). Further, the toxic effects of TNF have been shown to be modified by inhibition of cyclooxygenase (43). TNF may thus play an important role in mediating IL-2-induced injury.

Many of the effects of IL-2 administration are similar to those encountered following endotoxemia (40). The involvement of endotoxin is possible since the IL-2 used is E. coli derived. Furthermore, endotoxin stimulates the generation of several cytokines such as TNF and γ-interferon that are triggered also by IL-2. Nevertheless, endotoxin is an unlikely major cause of the observed changes induced by IL-2, since its concentration in the administered IL-2 preparation was below 0.06 ng/kg (3), whereas 0.5 to 1 ng/kg is required to produce a similar pulmonary response in sheep (40). Further, endotoxin directly damages endothelial cells (44), whereas IL-2 does not affect EC in vitro (3). Finally, boiling IL-2 at 100°C for 30 min, a regimen sufficient to inactivate IL-2 but not endotoxin, failed to produce any changes when infused into sheep (2 animals, data not shown).

The EXC of the IL-2 appears to be responsible at least in part for the pulmonary vasoconstriction and increased filtration pressure. However, this difficulty does not induce Tx generation and increased permeability (as the L/P protein ratio decreased while QL increased).

In summary, IL-2 administration leads to TxA₂ synthesis, derived from neutrophils, and the rapid onset of pulmonary dysfunction. Thromboxane mediates the pulmonary hypertension and the early phase in lung protein-rich lymph flow.

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ROLE OF TxA2 IN IL-2-INDUCED LUNG INJURY IN SHEEP

Role of Thromboxane in Interleukin 2-induced Lung Injury in Sheep


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