

## EFFECT OF INTERLEUKIN-2 ON MICROVASCULAR LIQUID AND PROTEIN TRANSPORT IN THE RAT SMALL INTESTINE

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### ABSTRACT

*Interleukin-2 (IL-2), a glycoprotein lymphokine derived from activated T-lymphocytes displays potent anti-cancer properties but its therapeutic use has been limited by generalized tissue swelling. To shed light on the mechanism underlying this potentially life-threatening edematogenic syndrome, recombinant IL-2 or an equal volume of control solution (excipient or 5% dextrose) was administered to 88 adult, male Sprague-Dawley rats. Initially, rats were injected with 50,000 Cetus units (equal to 300,000 I.U.) of IL-2 intraperitoneally, either one-time ("acute" rats) or every eight hours for two or seven days ("chronic" rats). Thereafter, under pentobarbital anesthesia, the main mesenteric lymph duct was isolated, incised, and measurements made of intestinal lymph flow ( $J_V$ ) and the total protein content and protein fractions in lymph (L) and plasma (P) (refractometry and agarose gel electrophoresis, respectively). Final measurements were also carried out after superior mesenteric vein constriction to assess filtration-independent L/P total protein "washdown." After IL-2,  $J_V$  and protein clearance ( $J_V \times L/P$ ) were increased ( $p < 0.001$ ) while lymph and plasma total protein content and protein fractionation were unchanged. Protein washdown was also maintained. These data are not only inconsistent with bulk "plasma leak" from damaged capillaries, but in conjunction with previously*

*demonstrated increased cardiac output and reduced systemic vascular resistance after IL-2 administration, the findings favor augmented microvascular surface exchange area from increased capillary perfusion as the primary mechanism underlying increased transcapillary liquid and protein flux. This conclusion conforms to the rapid reversal of edema in patients after cessation of IL-2 therapy.*

Interleukin-2 (or IL-2), a 15,000 dalton glycoprotein intimately involved in the biology of inflammation and the immune response, acts primarily as a stimulant of lymphocytes and of lymphokine-activated killer cell mitogenesis (1), and exhibits potent anti-growth activity against malignant tumors (1,2). A major limiting toxicity of IL-2 therapy, however, has been massive fluid retention, occasionally with life-threatening pulmonary edema. This complication has commonly been attributed to a "vascular leak syndrome" from disruption in microvascular integrity by mononuclear cells activated by IL-2 (so-called LAK cells) (3). Although LAK cells appear to damage endothelial cells *in vitro* (4), physiologic evidence of capillary disruption has not been clearly demonstrated in the intact host (5,6). In order to elucidate further the nature of IL-2's edematogenic side effect, we examined lymphatic transport of liquid and protein in the rat small intestine after a clinical dosing regimen of IL-2.

**Table 1**  
**Effect of IL-2 in Rats on Intestinal Lymph Flow ( $J_v$ ), Total Protein Concentration (tp) of Intestinal Lymph (L) and Plasma (P), the Lymph/Plasma Protein Ratio (L/P) and Protein Clearance ( $J_v \times L/P$ )**

Group	n	$J_v$ ( $\mu$ l/min)	$P_{tp}$ (mg/ml)	$L_{tp}$ (mg/ml)	L/P (tp ratio)	$J_v \times L/P$ ( $\mu$ l/min)
Control	33	12.1 $\pm$ 5.3*	58.8 $\pm$ 5.6	36.1 $\pm$ 9.6	.62 $\pm$ .15	7.0 $\pm$ 2.6
2 hour IL-2	11	21.0 $\pm$ 5.4†	64.5 $\pm$ 2.9	40.4 $\pm$ 3.2	.62 $\pm$ .12	13.0 $\pm$ 3.0†
2 day IL-2	16	25.8 $\pm$ 10†	58.7 $\pm$ 5.5	37.1 $\pm$ 6.9	.64 $\pm$ .10	16.2 $\pm$ 6.1†
7 day IL-2	28	25.4 $\pm$ 14†	60.0 $\pm$ 6.3	34.4 $\pm$ 8.1	.58 $\pm$ .14	14.4 $\pm$ 8.1†

\* Data shown as mean $\pm$ S.D.

†  $p \leq .001$  compared with control.

## MATERIALS AND METHODS

Eighty-eight adult Sprague-Dawley rats (body weight 368 $\pm$ 50gms; mean $\pm$ SD) were divided into groups receiving either human recombinant IL-2 (Cetus Corporation, Emeryville, CA) or a control solution of either IL-2 excipient (Cetus) or 5% dextrose in water. Rats were injected intraperitoneally either with a control solution or with 50,000 Cetus units (equal to 300,000 I.U.) one time ("acute" rats) or every 8 hours for 2 or 7 days ("chronic" rats). These time intervals were arbitrarily chosen to simulate short-term and somewhat longer term administration of IL-2 as used in treatment of human subjects. Following light anesthesia with intraperitoneal pentobarbital (~5mg), the main intestinal lymphatic paralleling the superior mesenteric artery was identified, incised, and a juxtaposed calibrated micro-pipet positioned to collect timed samples of lymph. Intestinal lymph flow ( $J_v$ ) was serially measured and total protein content of extremely small samples of both plasma and lymph determined by refractometry (A-O TS meter). In "acute" experiments,  $J_v$  was taken as the average lymph flow between 90 and 120 minutes after administration of IL-2 allowing 10-20 minutes for control (equilibration) time following preparatory manipulation. In "chronic" experiments,  $J_v$  was taken as the average lymph flow over 20-40 minutes after an equilibration time of 10-20

minutes following preparatory manipulation. Agarose gel separation of protein species of different molecular weights (albumin, alpha-1 and -2, beta, and gamma globulin) was also carried out in 18 rats (9 controls) on aliquots of plasma and lymph in 2-day chronic IL-2 rats to examine possible internal shifts in the distribution of protein fractions. Prior to termination of the experiment, increased mesenteric microvascular pressure was induced by superior mesenteric vein constriction in 43 rats (18 controls), and maximum dilution of mesenteric lymph protein content during heightened water flux was determined (filtration-independent protein "washdown") (7). Intestinal lymph protein clearance was calculated as  $J_v \times L/P$ . Data were subject to analysis of variance and student 2-tailed t test using SPSS (Statistical Package for the Social Sciences).

## RESULTS

Although no deaths occurred, many chronic IL-2 rats accumulated intraperitoneal fluid. Maximal intestinal lymph flow ( $J_v$ ) increased approximately two-fold overall (Table 1 and Fig. 1), but both the total protein content of plasma and lymph and the L/P protein ratio (shown in Table 1) and the protein fractions (Fig. 2) were unaltered after IL-2 therapy. As  $J_v$  increased, however, protein clearance or  $J_v \times L/P$  increased proportionately (Fig. 3).

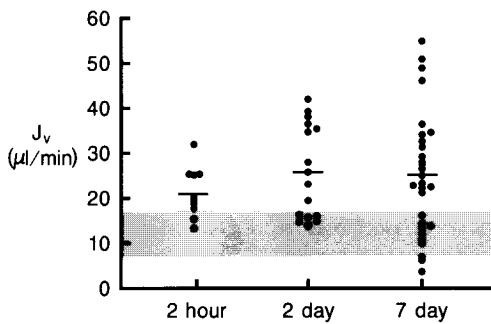


Fig. 1. Effect of acute (2 hour) and chronic IL-2 (2 and 7 days) IL-2 administration on intestinal lymph flow ( $J_v$ ) showing an approximate two-fold increase in fluid transport. Shaded area represents control values  $\pm$  S.D. Note that each point represents the mean of at least three separate timed measurements for volume flow of intestinal lymph to reach 0.1ml.

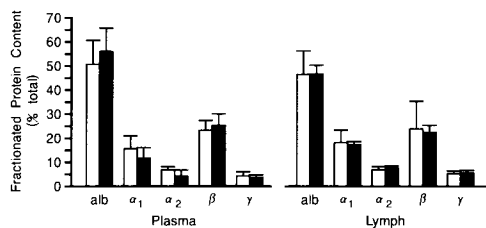


Fig. 2. Unaltered protein fractions (agarose gel electrophoresis) of plasma and intestinal lymph after 2-day IL-2 administration. The open bars represent controls (i.e., no IL-2) whereas the solid bars are the results after IL-2 administration. In the control group, total protein in plasma was  $56.1 \pm 5.5$  mg/ml whereas that in lymph was  $35.0 \pm 7.9$  mg/ml (mean  $\pm$  S.D.). In the IL-2 group, total protein was  $56.0 \pm 6.9$  mg/ml whereas that of lymph was  $34.1 \pm 7.8$  mg/ml, not significantly different from the control.

Nonetheless, induction of increased mesenteric hydrostatic pressure yielded prompt dilution ("washdown") of intestinal lymph protein as lymph flow increased in both acute and chronic IL-2 rats (Fig. 4). Among control rats, there was no difference in the results whether the "vehicle" (excipient) or 5% dextrose in water was administered.

## DISCUSSION

These experimental findings need to be viewed in the context of updated concepts regarding transcapillary liquid and

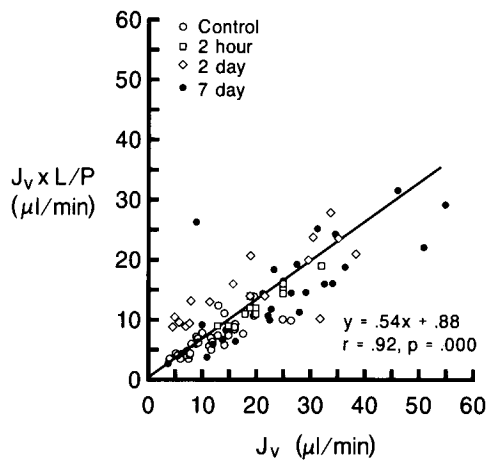


Fig. 3. Effect of IL-2 administration on lymph liquid ( $J_v$ ) and protein clearance ( $J_v \times L/P$ ). Note that as  $J_v$  increased, protein clearance increased proportionately. The geometric symbols represent individual rat responses in each designated group.

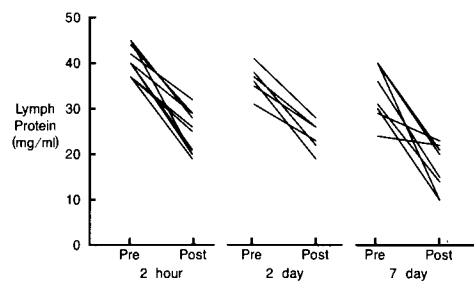


Fig. 4. Effect of increased mesenteric microvascular pressure (acute superior mesenteric venous constriction) on lymph protein "washdown." After IL-2 administration (2 hours, 2 days, 7 days) maximal dilution of lymph protein occurred within 20 minutes as intestinal lymph flow further increased. These data were similar to control rats after superior mesenteric vein constriction (total protein  $35.0 \pm 6.9$  before and  $20.2 \pm 4.7$  mg/ml after constriction).

protein movement and the pathogenesis of edema. According to the Starling hypothesis, edema or effusion arises from an imbalance between net capillary filtration and the rate of lymph absorption (8) or

$$\Delta IFV = K_d[(P_c - P_i) - \sigma(\pi_p - \pi_i)] - J_v$$

where IFV=interstitial fluid volume;  $K_t$ =a filtration coefficient that takes into account the nature of the capillary barrier and the perfused surface area;  $P_c$ =capillary hydrostatic pressure;  $P_t$ =tissue pressure;  $\sigma$ =solute reflection coefficient;  $\pi_p$ =plasma oncotic pressure;  $\pi_t$ =tissue oncotic pressure;  $J_v$ =net transcapillary liquid transport or lymph flow.

An imbalance in this equilibrium can arise either from a high output failure of the lymph circulation, where increased net capillary filtration outpaces lymph absorption, or, alternatively, in a low output failure where lymph absorption is primarily reduced (8).

The findings reported here in the rat small intestine, by others in the sheep lung (5,6,9) and by us previously in canine central lymph (10) consistently demonstrate an accelerated rate of lymph flow after IL-2 treatment. Greater lymph production may derive from a heightened transcapillary gradient of hydrostatic pressure (i.e.,  $\uparrow P_c - P_t$ ) or a reduced gradient of oncotic pressure (i.e.,  $\downarrow \pi_p - \pi_t$ ). The preponderance of clinical and experimental observations (5,6,9,11) favor that after IL-2 administration, microvascular pressure is not elevated, at least not early in the edema syndrome. Furthermore, as shown in the present experiments, plasma protein content (and therefore, plasma oncotic pressure) and intestinal lymph protein content (an approximation of tissue oncotic pressure) are both unaltered indicating that  $\pi_p - \pi_t$  is also unchanged. In this context, it is also noteworthy that the microvascular response is similar whether IL-2 is given as a single bolus or administered repeatedly over several days suggesting the effect is neither progressive nor cumulative.

The source of surplus lymph (and eventually edema fluid) after IL-2, therefore, most likely relates to an elevated capillary filtration coefficient ( $K_t$ ). Although  $K_t$  characteristically increases after disruption in capillary integrity as in thermal burns, plasma in this circumstance tends to leak in bulk from the vascular compartment resulting not only in increased  $J_v$  but also greater tissue oncotic

pressure (i.e.,  $\uparrow L/P$  protein) and a greater proportion of high molecular weight solute in lymph (i.e., less selective restriction) (12). Moreover, as microvascular integrity is compromised, protein "wash-down" no longer occurs when microvascular hydrostatic pressure is raised (i.e., capillaries are functionally fenestrated) (12). Note that this constellation of findings is not what occurs in the rat intestinal microcirculation after IL-2 treatment, where tissue oncotic pressure (total protein) and protein fractions are unaltered and protein washdown remains intact.

The expressions capillary "permeability" and "porosity" are clinically too often used imprecisely when applied to the complex phenomenon of liquid and solute movement in the microcirculation (12). An understanding of transcapillary protein movement ( $J_p$ ), for example, presumes knowledge of the nature of the capillary barrier, the surface area available for microcirculatory exchange, and transport mechanisms of "convection" and "diffusion" (14). These phenomenologic factors for microvascular solute transport are usually expressed as

$$J_p = J_v (1 - \sigma) \bar{C}_s + PS \bullet \Delta C$$

where  $J_p$ =transcapillary protein flux;  $J_v$ =volume flux or lymph flow;  $\bar{C}_s$  = the mean transmembrane protein concentration;  $P$ =the solute permeability of the capillary membrane;  $S$ =the membrane surface area;  $\Delta C$ =the protein concentration difference between plasma ( $C_p$ ) and lymph ( $C_l$ );  $\sigma$ =the reflection coefficient. The expression  $J_v(1-\sigma)C_s$  denotes the component of protein flux by filtration-convection (i.e., derived from microvascular hydrostatic pressure) and  $PS \bullet \Delta C$  expresses the component of protein flux by diffusion (i.e., from the transvascular protein concentration differential). In this context, the term "permeability-surface area product" or  $PS$  is crucial. Whereas *in vitro* effects of IL-2 on vascular endothelium have suggested direct injury from IL-2 activated LAK cells (4), our *in vivo* experimental findings are inconsistent with increased permeability ( $P$ ) as occurs with

endothelial disruption, "stretched pores," or a greater preponderance of larger filtering pores. Rather our data after IL-2 are most consistent with a greater capillary exchange surface area (S) as from recruitment of newly perfused capillaries with opening of more pores of a similar size. This interpretation conforms with the known hemodynamic changes produced by IL-2 (increased cardiac output, profound fall in systemic vascular resistance, and often systemic hypotension) (9,15) and also with the apparent rapid reversibility of effects including rapid resolution of edema after stopping IL-2 treatment (3,11). A "physiologic" rather than a "pathologic" or "toxic" effect of IL-2 also conforms to the observation that the microcirculatory response in the rat small intestine is neither progressive nor cumulative with repeated dosing.

Taken together, the findings suggest that IL-2 mediates an inflammatory response through a rapid yet reversible alteration in microcirculatory exchange of liquid, macromolecules, and immune cells. This powerful lymphokine accomplishes this task physiologically without incurring protracted damage to the endothelial lining. In this way, immunologic events are facilitated yet rapidly turned on and off without permanent tissue injury. In short, IL-2 at least in the intestinal microcirculation promotes a vascular "leak" not by capillary disruption, but through capillary recruitment and greater perfusion with an enhanced microvascular filtering surface area for solute and liquid exchange.

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#### REFERENCES

1. Morgan, DA, FW Ruscetti, RG Gallo: Selective *in vitro* growth of T lymphocytes from normal human bone marrows. *Science* 193 (1976), 1007-1008.
2. Rosenberg, SA, MT Lotze, YC Yang, et al: Experience with the use of high dose interleukin-2 in the treatment of 652 cancer patients. *Ann. Surg.* 210 (1989), 474-485.
3. Rosenstein, M, SE Ettinghausen, SA Rosenberg: Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin 2. *J. Immunol.* 137 (1986), 1735-1742.
4. Damle, NK, LV Doyle, JR Bender, et al: Interleukin 2-activated human lymphocytes exhibit enhanced adhesion to normal vascular endothelial cells and cause their lysis. *J. Immunol.* 138 (1987), 1779-1785.
5. Harms, BA, AC Pahl, TH Pohlman, et al: Effects of interleukin-2 on pulmonary and systemic transvascular fluid filtration. *Surgery* 106 (1989), 339-346.
6. Klausner, JM, N Morel, IS Paterson, et al: The rapid induction by interleukin-2 of pulmonary microvascular permeability. *Ann. Surg.* 210 (1989), 119-128.
7. Granger, DN, AE Taylor: Permeability of intestinal capillaries to endogenous macromolecules. *Am. J. Physiol.* 238 (1980), H457-H464.
8. Witte, CL, MH Witte: Lymphatics in pathophysiology of edema. In *Experimental Biology of the Lymphatic Circulation*, Johnston, MG (Ed.), Elsevier, Toronto (1985), 165-188.
9. Jesmok, GJ, RA Gunther: *In vivo* biology of recombinant interleukin-2 infusion in sheep: Cardiopulmonary manifestations of an intravascular immune-inflammatory response. *Inflammation* 13 (1989), 267-284.
10. Stuntz, ME, J Champsi, M Witte, et al: Lymphogague effect of acute and chronic interleukin-2 in the dog. In *Symposium Proceedings - XIIth*, Proc. XIIth Int'l. Cong. of Lymphology, Nishi, M, S Uchino, S Yabuki (Eds.), Elsevier, Amsterdam:New York:Oxford (1990), 511-513.
11. Lotze, MT, L Yvedt, YL Matory, et al: Clinical effects and toxicity of interleu-

- kin-2 in patients with cancer. *Cancer* 58 (1986), 2764-2772.
12. Taylor, AE, DN Granger: Exchange of macromolecules across the microcirculation. In *Handbook of Physiology, Section 2, The Cardiovascular System*. Renkin EM, CC Michell CC (Eds.), APS Bethesda, Maryland (1984), 467-520.
  13. Curry, F-RE: Determinants of capillary permeability: A review of mechanisms based on single capillary studies in the frog. *Circ. Res.* 59 (1986), 367-380.
  14. Renkin, EM, WL Joyner, CH Sloop, et al: Influence of venous pressure on plasma-lymph transport in the dog's paw. *Convective and dissipative mechanisms. Microvas. Res.* 14 (1977), 191-204.
  15. Glauser, FL, GG DeBlois, DE Berchard, et al: Cardiopulmonary effects of recombinant interleukin-2 infusion in sheep. *J. Applied. Physiol.* 64 (1988), 1030-1037.

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