American Journal of Pathology, Vol. 135, No. 1, July 1989

Protein Synthesis Dependent and Independent Mechanisms of Neutrophil Emigration

Different Mechanisms of Inflammation in Rabbits Induced by Interleukin-1, Tumor Necrosis Factor Alpha or Endotoxin Versus Leukocyte Chemoattractants

Myron I. Cybulsky, Donna J. McComb, and Henry Z. Movat

Departments of Pathology and Immunology, University of

Inflammation constitutes the body's principal mode of defense against infection and other harmful agents. Neutrophil leukocytes are the primary effector cells in this process. The role of protein synthesis in neutrophil emigration into acute inflammatory lesions was examined. Local intradermal injections of actinomycin D, cycloheximide or puromycin could inhibit in a dose- and time-dependent manner neutrophil emigration induced by interleukin-1, tumor necrosis factor alpha or endotoxin, but not by the leukocyte chemoattractants C5a des arg (zymosan-activated plasma), n-formyl-methionyl-leucyl-phenylalanine or leukotriene B_4. Maximal inhibition, measured at the time of peak emigration, was greater than 90%. The onset of neutrophil emigration induced by the cytokines or by endotoxin was delayed by 30 to 60 minutes in comparison to the leukocyte chemoattractants. These results demonstrate at least two mechanisms of neutrophil emigration: one with a slower onset and dependence on local RNA transcription and translation and the other rapid in onset and independent of protein synthesis. (Am J Pathol 1989, 135:227–237)

Neutrophil emigration from the blood into tissues through the walls of postcapillary venules and small veins is the hallmark of acute inflammation. Neutrophils first adhere to endothelial cells, then undergo diapedesis and migrate into extravascular tissues. Although this phenomenon has been studied for over 100 years, it is only in the last decade that a quantitative *in vivo* approach has been developed and systematically used.[1,2] Radiolabeled blood neutrophils accumulate rapidly in intradermal inflammatory sites. The similarity in the kinetics of neutrophil emigration, irrespective of the nature of the inflammatory stimuli, whether bacteria,[3] endotoxin (lipopolysaccharide, LPS) (4), leukocyte chemoattractants (lipid, protein or peptide) (4) or cytokines,[5,6] suggests a highly regulated process that is not simply dependent on the local diffusion of inflammatory stimuli. This notion is also supported by the tachyphylaxis (desensitization) experiments of Colditz and Movat.[7–9] In these studies neutrophil accumulation was attenuated only in intradermal sites which were previously injected with the same inflammatory stimulus, and was normal in sites injected with most other stimuli.

Vascular endothelial cells when exposed to inflammatory stimuli may play a key role in regulating leukocyte emigration by becoming more adherent for leukocytes. This concept is based on the early observations of Metchnikoff[10] and of Clark and Clark[11]; more recently on tachyphylaxis experiments[7–9] and on *in vitro* studies.[12–15] In response to stimulation by LPS, interleukin-1 (IL-1), tumor necrosis factor alpha (TNF) or phorbol myristate acetate, cultured vascular endothelial cells become hyperadhesive for neutrophils.[12–15] This phenomenon appears to be mediated in part by inducible endothelial cell surface proteins and can be blocked by protein synthesis inhibitors. To date two such proteins designated ELAM-1[16,17] and ICAM-1,[18] have been identified.

The regulation of neutrophil emigration may also be dependent on the production of inflammatory mediators

This work was supported by the Medical Research Council of Canada, Grant MT-1251. Dr. Cybulsky is a postdoctoral fellow of the Medical Research Council of Canada. Dr. Movat is a career investigator of the Medical Research Council of Canada.

Accepted for publication April 7, 1989.

Requests should be addressed to Dr. Henry Z. Movat, Department of Pathology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Correspondence to Myron Cybulsky, Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St., Boston, MA 02115.

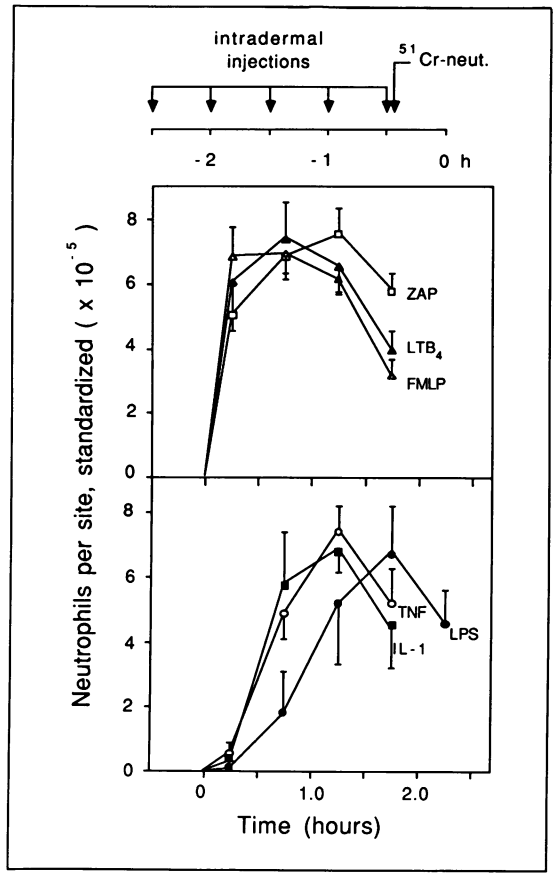

Figure 1. *Kinetics of neutrophil emigration. Separate intradermal sites were injected with inflammatory stimuli at the various times indicated by the arrow heads (top) and the rate of neutrophil accumulation was quantitated during the final 30 min with ^{51}Cr-labeled blood-derived neutrophils. The means and SEM are plotted at the midpoint of each 30 min interval. Variability between rabbits was minimized by standardizing the number of neutrophils accumulated in each site to 10^6 circulating neutrophils/ml (neutrophils per site, standardized). Middle: leukocyte chemoattractants-ZAP 33%, 0.2 ml/site (approx. 3 × 10^{-11} moles of C5a des arg/site), (□, n = 15), FMLP 10^{-10} moles/site (△, n = 12) and LTB$_4$ 10^{-9} moles/site (▲, n = 9). Bottom: IL-1 10^{-13} moles/site (■, n = 12), TNF 10^{-10} moles/site (○, n = 9) and LPS 10^{-13} moles/site (1.2 ng/site) (●, n = 12).*

by tissue macrophages, mast cells or smooth muscle cells. In the case of cytokines and some neutrophil chemoattractants, their production requires protein synthesis. We have therefore investigated the role of protein synthesis on neutrophil emigration in an *in vivo* rabbit model. The roles of transcriptional and translational events in neutrophil emigration were examined, using actinomycin D (AD), cycloheximide (CH) or puromycin (PM) injected directly into acute inflammatory sites. The results indicate that emigration in response to cytokines or LPS has a slower onset and can be blocked by inhibitors of protein synthesis, while emigration in response to leukocyte chemoattractants is rapid in onset and independent of protein synthesis. Preliminary findings were presented in abstract form.[19]

Methods

Quantitation of Neutrophil Emigration

New Zealand albino rabbits weighing 3–4 kg were used. Neutrophil emigration (adherence to vascular endothelial cells followed by diapedesis) was estimated by measuring the accumulation (intra and extravascular) of ^{51}Cr-labeled neutrophils into inflammatory sites.[2,20] Allogeneic neutrophils were isolated from blood by sedimentation and density gradient centrifugation. After labeling with $Na_2{}^{51}CrO_4$ (New England Nuclear, Lachine, Quebec), a ^{51}Cr-labeled erythrocyte contaminant was removed by resedimentation and the labeled neutrophils were injected i.v. into experimental rabbits. Blood samples were obtained after 15 min (the midpoint of the period during which neutrophil emigration was measured) and blood neutrophil specific activity was determined. The number of host neutrophils accumulated in each site was determined by dividing the ^{51}Cr-radioactivity per site by the blood neutrophil specific activity. Interanimal variability was standardized to 10^6 circulating neutrophils per ml of blood.[20] Previous studies have validated this approach by demonstrating that ^{51}Cr-labeled allogeneic neutrophils emigrate into pleural exudates with the same efficiency as unlabeled neutrophils of the host[20] and in response to an intravenous injection of LPS marginate identically to the host's neutrophils.[21]

Injection Protocols

The rate of neutrophil accumulation (kinetics) was determined by injecting different intradermal sites with an inflammatory stimulus every 30 min, and allowing ^{51}Cr-labeled neutrophils to circulate during the final 30 min. In other experiments neutrophil accumulation was measured during the 30 min interval when the rate of accumulation was maximal, or immediately after the injection of the inflammatory stimulus. For each measurement three sites were injected per rabbit. Protein synthesis inhibitors were injected intradermally and the timing of these injections is explained in the results section for each experiment.

Reagents

Stocks of inflammatory stimuli were diluted to working concentrations with pyrogen-free saline (Baxter-Travenol Labs, Toronto). A volume of 0.2 ml was injected per site and approximately equipotent doses were used, as was determined previously in dose-response studies.[4,5] Human recombinant IL-1 alpha (3 × 10^7 U/mg protein, lot no

14992-50-93) was a gift from Drs. Peter T. Lomedico and Alvin S. Stern, Hoffmann-LaRoche (Nutley, NJ). Human recombinant TNF alpha (5×10^7 U/mg protein) was provided by Genentech (South San Francisco, CA). LPS was derived from *E. coli* 055:B5, lot 682197 (Difco Labs, Detroit, MI) and its molarity was estimated using a m.w. of 12,000. N-Formyl-methionyl-leucyl-phenylalanine (FMLP) (Sigma Chemical Co., St. Louis, MO) was dissolved at 10^{-2} M in dimethyl sulfoxide and diluted with saline. Leukotriene B_4 (LTB_4) (100 ug/ml methanol) was a gift of Dr. J. Rokach, Merck Frosst Canada, Inc. (Dorval, Quebec). Zymosan-activated rabbit plasma (ZAP) was prepared, as described previously[22] and the molarity of C5a des Arg was estimated assuming complete conversion of C5.

Actinomycin D (AD) (Sigma Chemical Co., St. Louis, MO) was dissolved in methanol and was stored at −20° C. On the day of the experiment the AD solution was diluted with saline to the working concentration. The final concentration of methanol in a 5×10^{-4} M AD solution was 0.1%. Cycloheximide (CH) and Puromycin (PM) (Sigma Chemical Co., St. Louis, MO) were dissolved in saline immediately prior to use.

Statistics

Inflammatory lesions were injected in triplicate in each rabbit. The means and SEM were calculated based on the values of each intradermal site and significant differences were determined using a two-way analysis of variance.

Results

Onset of Neutrophil Emigration

The rates of neutrophil accumulation were determined for sequential 30 min time intervals following the injections of equipotent doses of various inflammatory stimuli (Fig. 1, top). A difference was found in the rapidity of the onset of neutrophil accumulation between the leukocyte chemoattractants (Fig. 1, middle) and cytokines or LPS (Fig. 1, bottom). Neutrophil accumulation in response to leukocyte chemoattractants was rapid. In the first 30 min after injections of ZAP, LTB_4 or FMLP, the rate of neutrophil accumulation was greater than 65% of maximal (at 30–90 min). In contrast, the onset of neutrophil accumulation after injections of IL-1, TNF or LPS was slow and during the first 30 min the rate of accumulation was 4%, 11% and 1% respectively of the maximal (at 60–120 min).

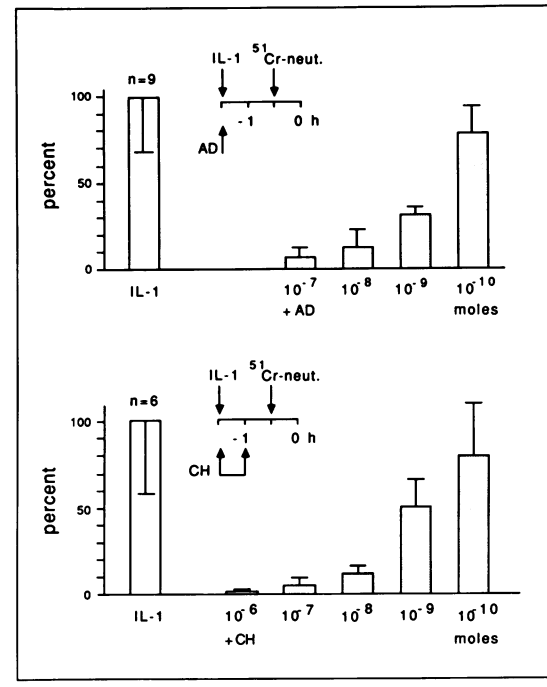

Figure 2. *Dose-dependence of protein synthesis inhibitors. The effects of increasing doses of AD (top) or CH (bottom) on IL-1-induced neutrophil accumulation were measured. Intradermal sites were injected with IL-1 (10^{-13} moles/site) at −1.5 hr and also with increasing doses of AD at −1.5 hr (top), or at −1.5 and at −1.0 hr with CH (bottom). CH required 2 injections since it was short acting. Neutrophil accumulation was measured at −0.5 to 0 hr (1.0–1.5 hr after the injection of IL-1, when the rate of accumulation was maximal; see Fig 1). The means and SEM for triplicate injections in 3 rabbits, top (n = 9) and 2 rabbits, bottom (n = 6) are plotted as % of control (IL-1 alone = 100%).*

Dependence of Inhibition of Neutrophil Emigration on the Dose of Protein Synthesis Inhibitors

The effects of intradermally injected AD, CH or PM on neutrophil accumulation were examined at the 30 min interval when the rate of accumulation was maximal (Fig. 1). Figure 2 illustrates the effects of increasing doses of AD or CH on IL-1-induced neutrophil emigration. In this case, as with LPS or TNF (not shown), increasing doses of protein synthesis inhibitors progressively diminished neutrophil accumulation. Injections of 10^{-9} moles/site of either AD or CH suppressed emigration by 50% or greater, and 10^{-7} moles/site by 90% or greater (Fig. 2).

Dependence of Inhibition of Neutrophil Emigration on the Timing of Protein Synthesis Inhibitor Injections

IL-1 and LPS were the inflammatory stimuli and neutrophil accumulation was measured during the 30 min interval

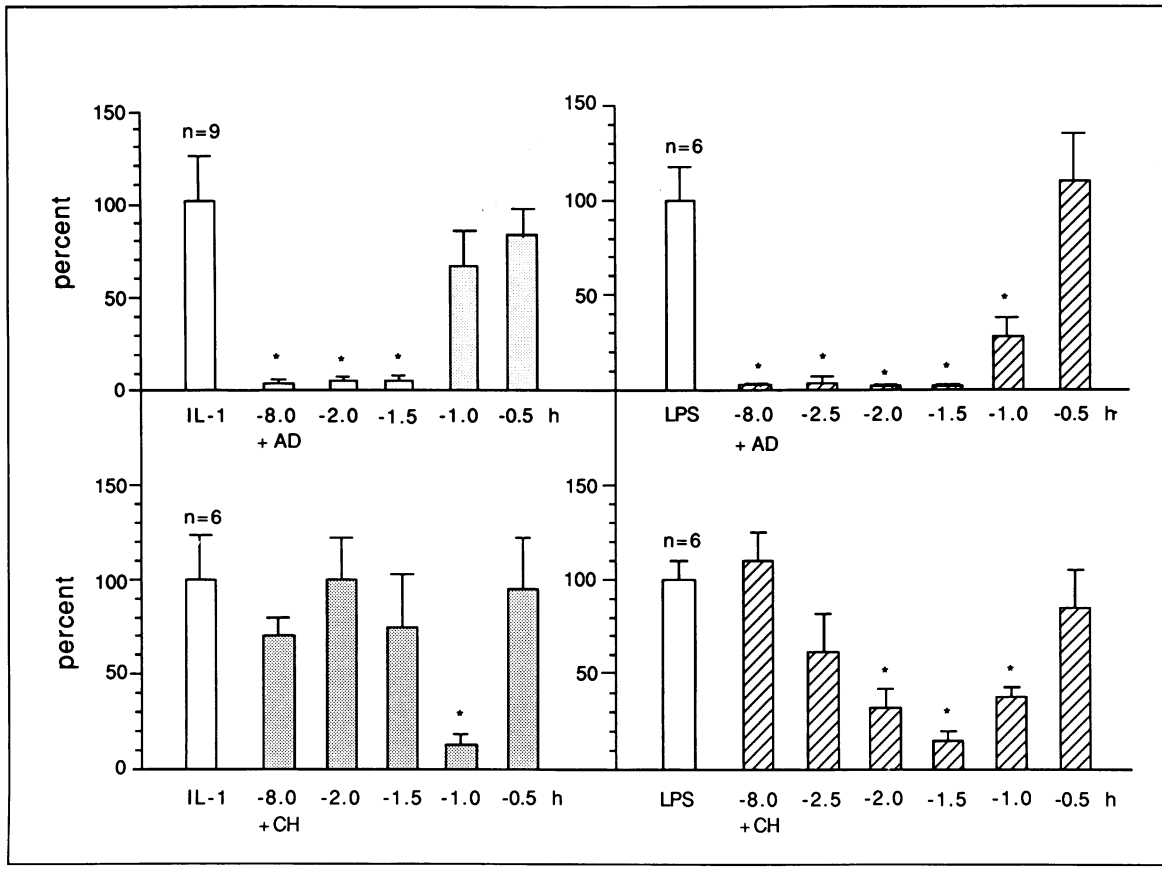

Figure 3. *Time-dependence of AD and CH. Intradermal sites were injected at −1.5 hr with IL-1 10^{-13} moles/site (left) or at −2 hr with LPS 10^{-13} moles/site (right). AD 10^{-7} moles/site (top) or CH 10^{-7} moles/site (bottom) was injected at the time indicated on the abscissa. Neutrophil accumulation was measured at −0.5 to 0 hr and the means and SEM are expressed as % of control. Significant differences (* = P < 0.05) are indicated.*

when the rate of accumulation was maximal (at 1–1.5 hr for IL-1 and at 1.5–2 hr for LPS, see Fig. 1). AD or CH were injected at different times into these dermal sites and their effects on neutrophil accumulation were determined (Fig. 3). AD could inhibit neutrophil accumulation even when injected 6.5 or 6 hours prior to IL-1 or LPS (Fig. 3, top). In contrast, CH had no effect when injected prior to IL-1 or LPS (Fig. 3, bottom), and even when injected simultaneously with IL-1 (−1.5 hr) minimal inhibition was observed (Fig. 3, bottom left). Significant diminution of neutrophil accumulation was observed only when CH was injected 30 min after IL-1 (−1 hr). With LPS (Fig. 3, bottom right), CH inhibited neutrophil emigration when it was injected simultaneously with LPS (−2 hr), 30 min after LPS (−1.5 hr) or 1 hr after LPS (−1 hr). Based on these experiments, it was determined that CH would be most efficacious if 2 injections were given; the first simultaneously with the inflammatory stimulus and the second 30 min later.

PM was even shorter acting than CH, in that a single injection, irrespective of the timing, would not significantly diminish either IL-1- or LPS-induced neutrophil accumulation (not shown). For significant inhibition injections of PM were required at every 30 min.

Different Effects of Protein Synthesis Inhibitors on Neutrophil Emigration in Response to LPS, IL-1 or TNF Versus Leukocyte Chemoattractants

Inhibitors of protein synthesis could decrease neutrophil accumulation elicited by LPS (Fig. 4), IL-1 (Fig. 5) or by TNF (Fig. 6) by 85% of control or greater. As demonstrated earlier, AD was long acting and was effective either when injected together with or prior to LPS or cytokines. In contrast, prior injections of CH or PM were not effective, and multiple injections of these inhibitors simultaneously with and following LPS or cytokines were required (Figs. 4–6). When protein synthesis inhibitors were injected 30 min prior to killing the rabbits (1.5 hr after the injection of LPS and 1 hr after IL-1 or TNF) they did not have a significant effect. This suggests that the majority

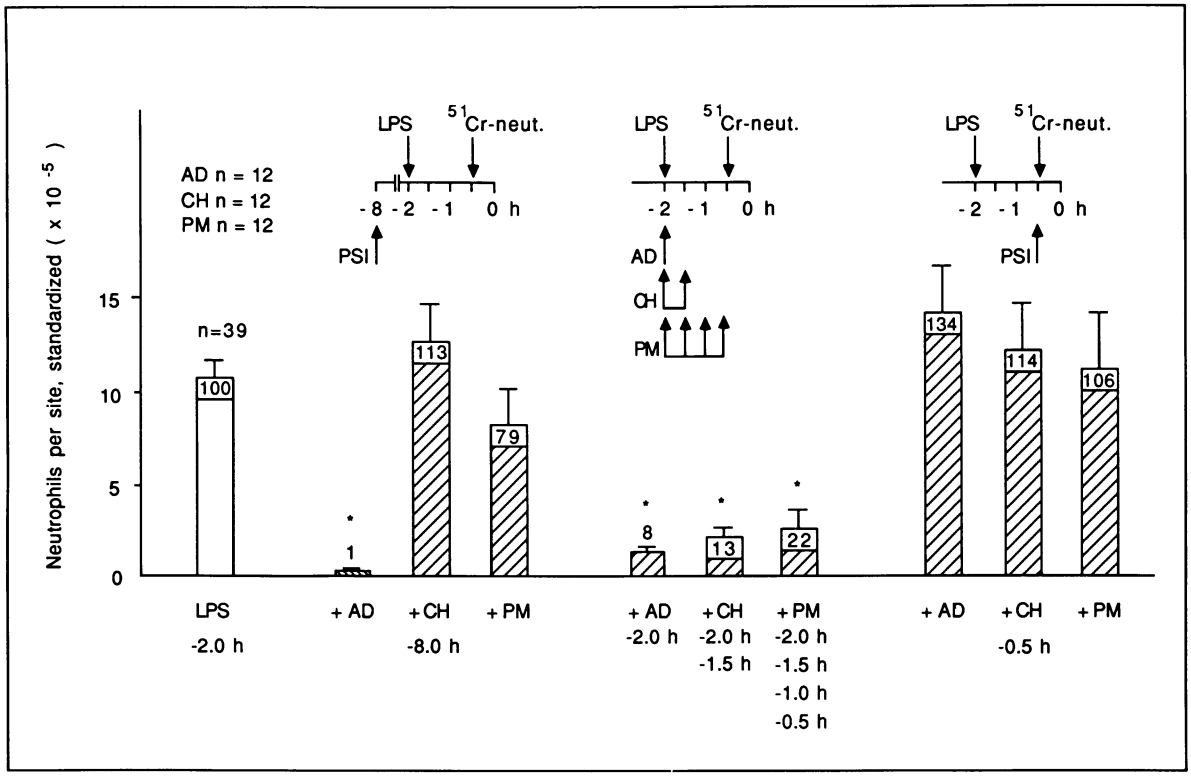

Figure 4. *Inhibition of LPS-induced neutrophil accumulation by protein synthesis inhibitors. Intradermal sites were injected with LPS 10^{-13} moles/site at −2 hr. These sites were also injected with protein synthesis inhibitors (PSI), as indicated at the top of the graph, at −8 hr (left), together with and following LPS (middle) or at −0.5 hr (right). For each intradermal site, the total dose of AD, CH or PM was the same, whether given in a single injection or as multiple, equal injections (10^{-7} moles/site of AD, 2×10^{-7} moles/site of CH or 2 injections, each 10^{-7} moles, and 3×10^{-5} moles/site of PM or 4 injections, each 7.5×10^{-6} moles). Neutrophil accumulation was quantitated at −0.5 to 0 hr and the means and SEM are plotted. The % of control (LPS alone = 100%) is determined and significant differences (* = $P < 0.05$) are indicated.*

of local protein synthesis induced by LPS or cytokines had already occurred prior to these injections.

Irrespective of the timing of their injections, protein synthesis inhibitors did not significantly diminish the neutrophil accumulation by ZAP (C5a des Arg), FMLP or LTB_4 (Table 1). Two time periods were examined: the initial 30 minutes after the injection of a leukocyte chemoattractant and the 30 min interval when the rate of emigration was maximal. The early period was also examined because unlike with LPS or cytokines, chemoattractants elicited a rapid neutrophil accumulation (Fig. 1).

We gave consideration to the possibility that intradermal sites when injected with LPS or cytokines combined with an inhibitor of protein synthesis become incapable of supporting neutrophil emigration. This could be the result of functional changes in endothelial or other autochthonous cells, or secondary to local disturbances of blood flow e.g. stasis or microthrombosis, which would prevent neutrophil accessibility to the inflammatory site. We therefore examined these possibilities. IL-1 and AD or IL-1 and CH treated sites were injected also with FMLP. The magnitude of neutrophil accumulation into these sites was comparable to skin sites injected only with FMLP (Fig. 7). This demonstrates that protein synthesis inhibitors, even when combined with IL-1 do not abrogate local neutrophil recruitment by FMLP, a stimulus, which presumably uses a different mechanism from IL-1.

Inflammatory sites were examined microscopically and during the experimental period there was no evidence of stasis, microthrombosis or of necrosis (Fig. 8). Moreover, morphology demonstrated emigration of neutrophils into extravascular tissues, which cannot be distinguished from intravascular accumulation by a radiometric assay. Tissue necrosis was evident only after 24 hours in sites injected with AD alone or together with cytokines or LPS.

Effect of CH on the Kinetics of IL-1-Induced Neutrophil Emigration

The inhibition of neutrophil accumulation by CH was dose-dependent (Fig. 2), short acting and reversible (Fig. 3). If this effect was because of a temporary block of RNA translation by CH, it should be reversible, provided that relevant messenger RNA remained stable. This hypothe-

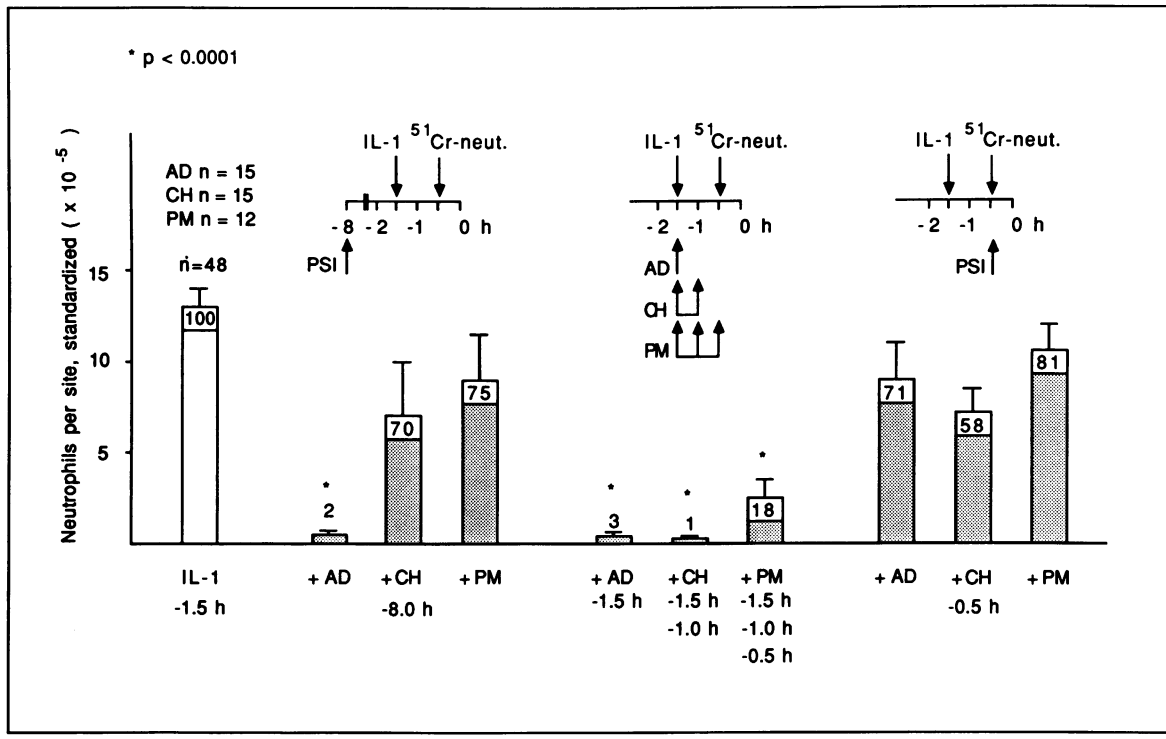

Figure 5. *Inhibition of IL-1-induced neutrophil accumulation by protein synthesis inhibitors. Intradermal sites were injected with IL-1 10^{-13} moles/site at −1.5 hr. The timing of AD, CH or PM injections is indicated at the top (AD = 10^{-7} moles/site, CH = 2×10^{-7} moles/site or 2 injections, each 10^{-7} moles and PM = 2.25×10^{-5} moles/site or 3 injections, each 7.5×10^{-6} moles/site). Neutrophil accumulation and the % of control (IL-1 alone) are plotted and significant differences (* = P < 0.05) are indicated.*

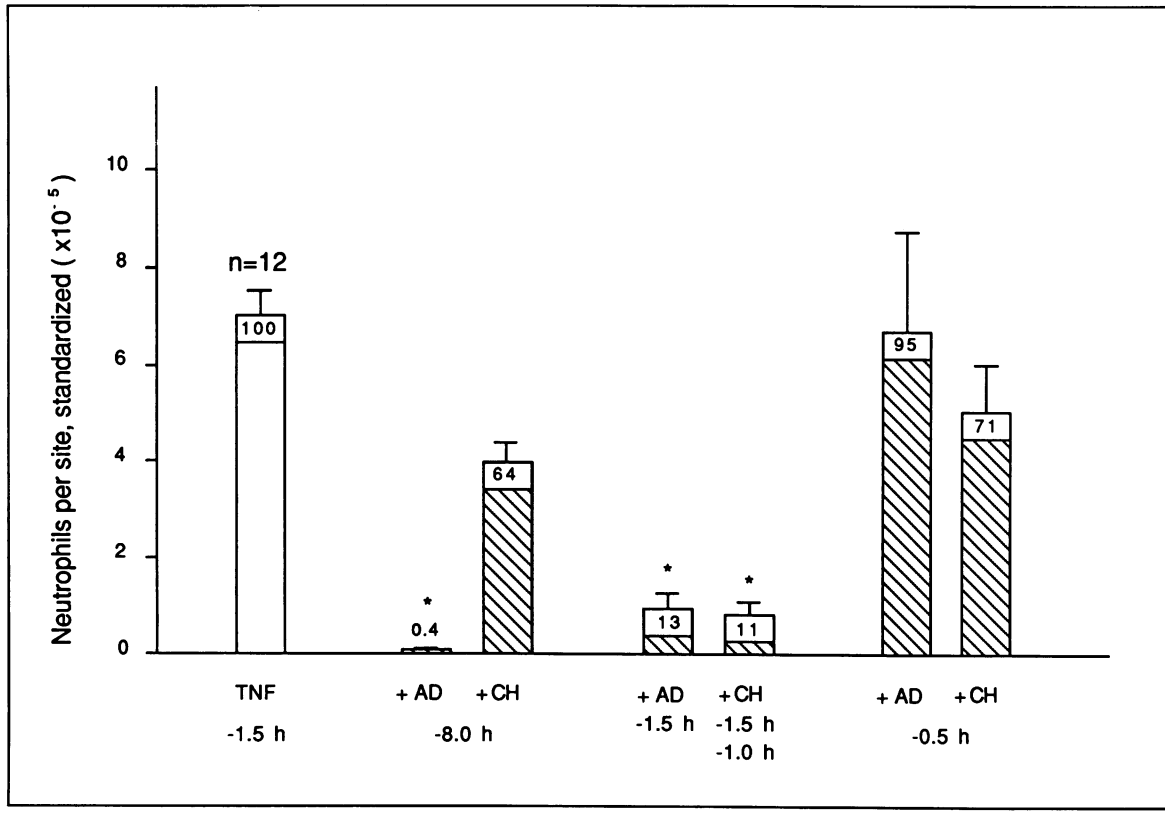

Figure 6. *Inhibition of TNF-induced neutrophil accumulation by protein synthesis inhibitors. The experimental protocol is identical to Fig 5, except that TNF 10^{-10} moles/site was the inflammatory stimulus.*

Table 1. *Effect of AD or CH on Neutrophil Accumulation Elicited by ZAP, FMLP or LTB_4*

Inflammatory stimulus	Neutrophils accumulated per site, standardized ($\times 10^{-5}$)				
				+AD*	+CH
(injection time) n = 12	Alone	+AD (−0.5 h)	+CH (−0.5 h)	(injected with the inflam. stimulus)	
ZAP† (−1.5 h)	8.3 ± 0.7	12.4 ± 1.0	10.7 ± 1.2	6.4 ± 0.6	7.3 ± 1.0
ZAP (−0.5 h)	4.9 ± 0.5	7.2 ± 1.1	4.6 ± 0.9		
FMLP (−2.0 h)	3.4 ± 0.4	4.9 ± 0.5	6.2 ± 0.8	4.2 ± 0.6	4.7 ± 0.6
FMLP (−0.5 h)	6.2 ± 0.6	8.9 ± 1.3	5.1 ± 0.6		
LTB_4 (−2.0 h)	6.8 ± 0.7	7.8 ± 1.1	7.5 ± 1.1	6.9 ± 1.0	7.3 ± 1.7
LTB_4 (−0.5 h)	8.0 ± 1.0	9.5 ± 1.9	8.8 ± 0.9		

* Doses and timing of protein synthesis inhibitor injections, same as in Figs. 4–6.
† Doses same as in Fig. 1.
Abbreviations: AD, actinomycin D; CH, cycloheximide; FMLP, n-formyl-methionyl-leucyl-phenylalanine; LTB_4, leukotriene B_4; PM, puromycin; ZAP, zymosan activated plasma.

sis was tested by examining the effects of CH on the rate of IL-1-induced neutrophil accumulation (Fig. 9). Intradermal sites were sequentially injected either with IL-1 alone, or with IL-1 and CH. The rate of neutrophil accumulation was measured with ^{51}Cr-labeled neutrophils, as shown in the top panel of Fig. 1. A 1.5 hour delay or right shift was observed in the kinetics of IL-1 plus CH (Fig. 9) yet the kinetic profile was similar to IL-1 alone. These findings support the hypothesis that inhibition of IL-1-induced neutrophil emigration by CH was the result of reversible blockade of RNA translation.

Discussion

Although neutrophil emigration in response to many different inflammatory stimuli is similar,[4] we now demon-

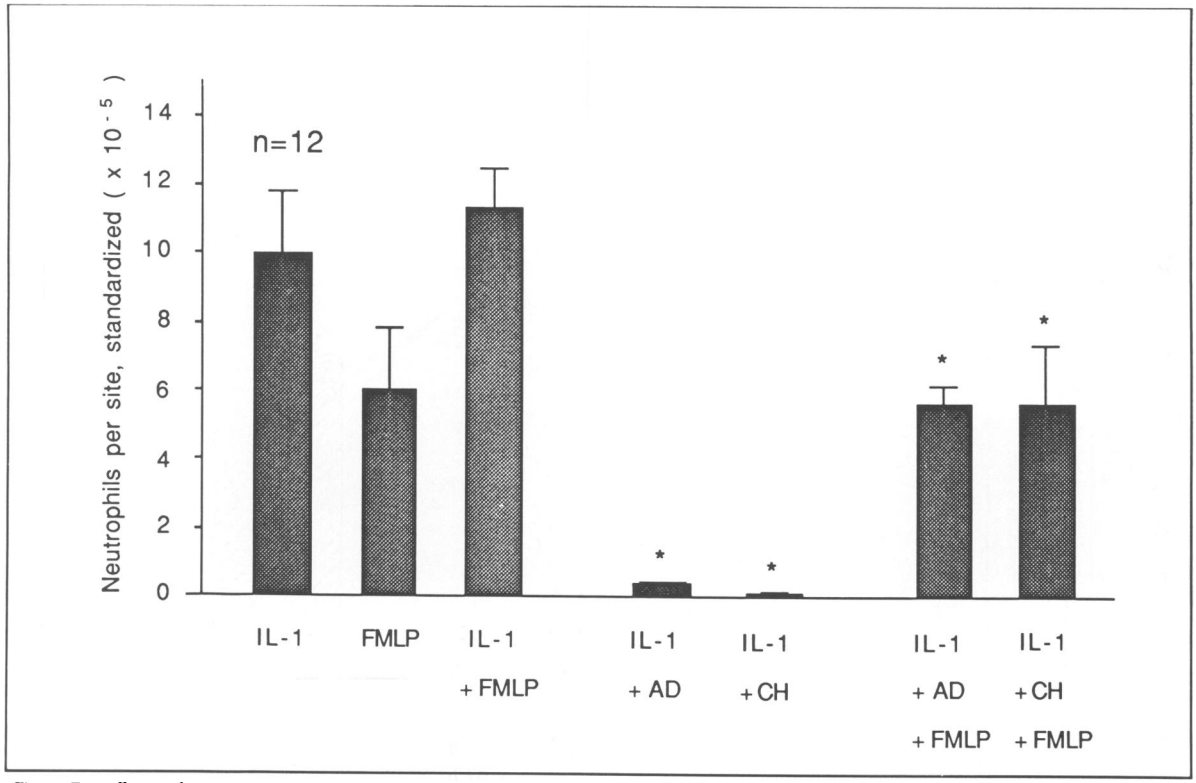

Figure 7. *Effects of AD or CH on neutrophil accumulation induced by injections of IL-1 + FMLP. IL-1 (10^{-13} moles/site) and FMLP (10^{-10} moles/site) were injected at −1.5 hr. AD (10^{-7} moles/site) was injected also at −1.5 hr and CH was injected at −1.5 and at −1.0 hr (10^{-7} moles/injection). Neutrophil accumulation was measured at −0.5 to 0 hr. Both AD and CH significantly diminished IL-1-induced neutrophil accumulation (middle), as compared to IL-1 alone (* = $P < 0.05$). IL-1 + FMLP injected with AD or CH (right) differed significantly from IL-1 + FMLP, but did not differ significantly from FMLP injected alone.*

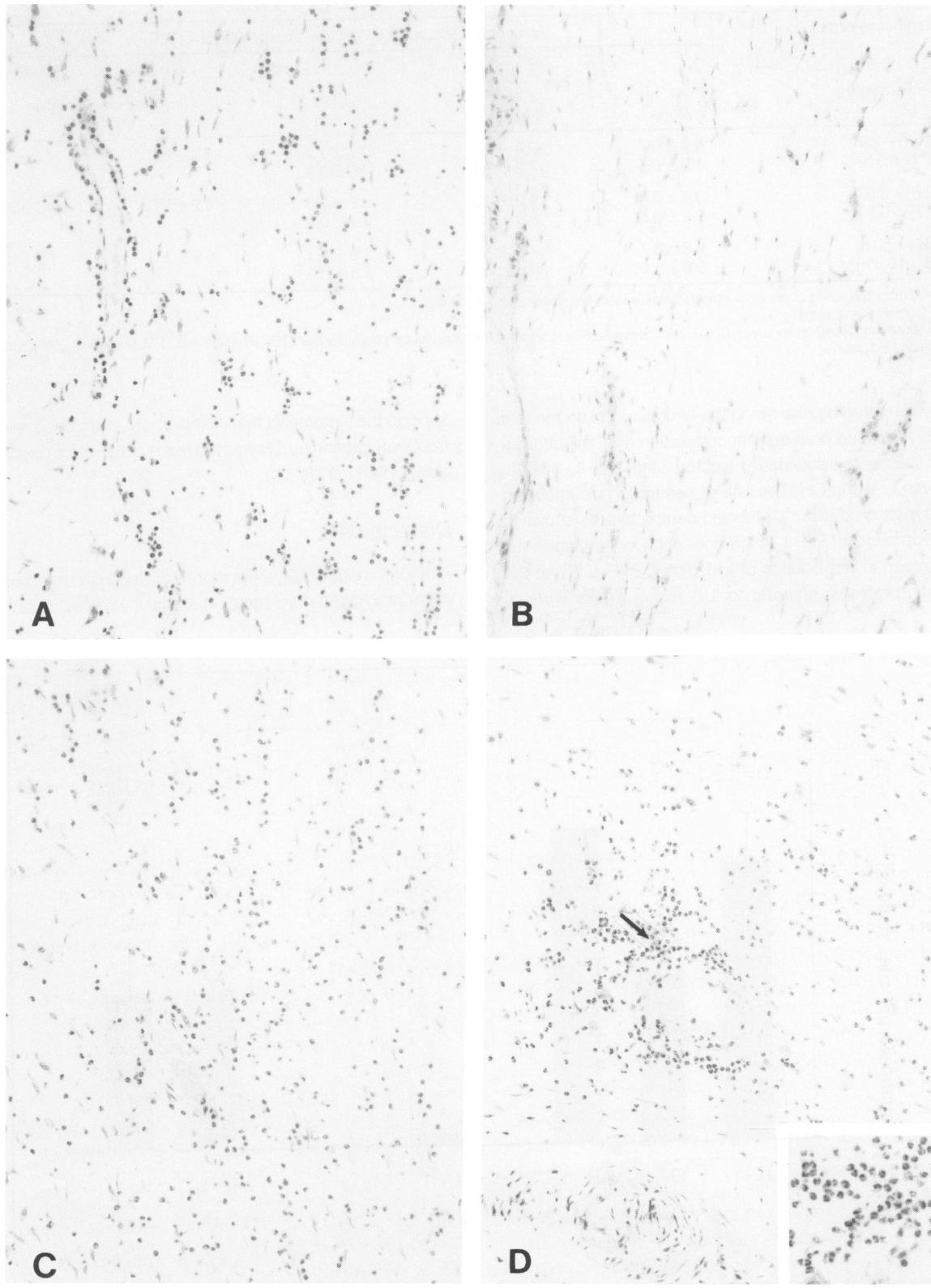

Figure 8. *Histology of inflammatory lesions. Fig 8A is representative of a 4 hr lesion induced by LPS (10 ug/site) and Fig 8B by LPS plus AD (10^{-7} moles/site). Fig 8C illustrates a site injected with ZAP (100%, 0.2 ml/site) and Fig 8D ZAP plus AD. While the inhibitor prevented the accumulation of leukocytes induced by LPS (Fig 8B), it had no effect at sites injected with ZAP (Fig 8D). The inset in Fig 8D shows at higher magnification the area indicated by the arrow, demonstrating that the accumulated leukocytes are neutrophils.*

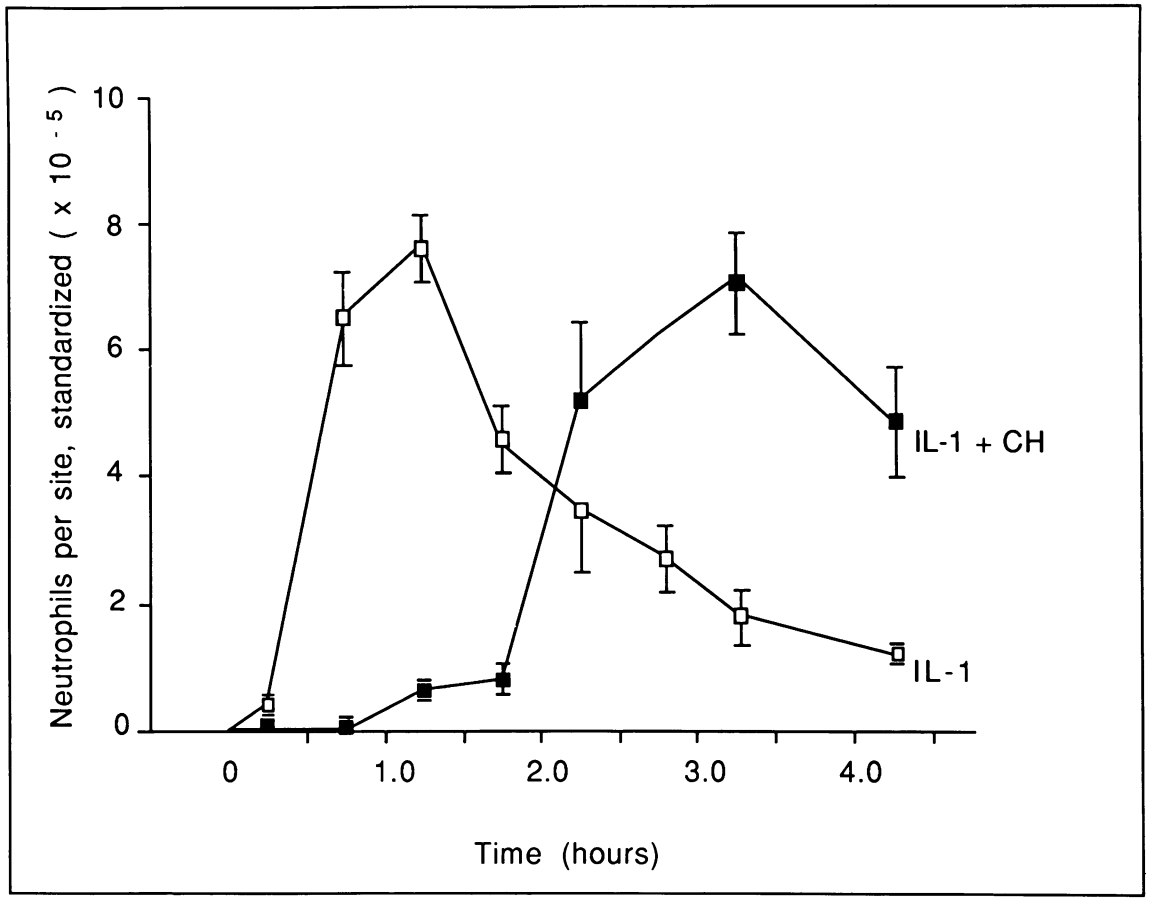

Figure 9. *Effect of CH on the kinetics of IL-1-induced neutrophil accumulation. Intradermal sites were sequentially injected with IL-1 10^{-13} moles/site (□) or IL-1 + CH 10^{-7} moles/site (●). The latter sites also received a second injection of CH after 30 min. Neutrophil accumulation was determined during the final 30 min and the rate (mean and SEM, n = 9) is plotted, as in Fig 1, at the midpoint of each 30 min period.*

strate that the onset of emigration elicited by leukocyte chemoattractants is more rapid than with cytokines or LPS. This observation suggests that different mechanisms may be operating. The 30–60 min delay in the onset of neutrophil emigration in response to cytokines or LPS may represent the time required for the synthesis of proteins which mediate this process. Studies with inhibitors of protein synthesis were undertaken in order to examine the role of RNA transcription and translation in neutrophil emigration. The results indicate that neutrophil accumulation elicited by IL-1, TNF or LPS, in contrast to leukocyte chemoattractants, can be blocked by greater than 85% with AD, CH or PM injected directly into the inflammatory site. This effect is dose- and time-dependent. The effects of AD (an irreversible blocker of RNA transcription) were prolonged, while of CH and PM (reversible blockers of RNA translation) were short acting and thus multiple injections of CH and PM were necessary to effectively suppress neutrophil emigration. When CH was injected simultaneously with IL-1, the kinetics of neutrophil emigration were delayed by 60–90 min (Fig. 9), in keeping with the notion that CH blocks RNA translation reversibly. Recently Rampart and Williams have also demonstrated that AD and CH could inhibit IL-1- but not chemoattractant-induced neutrophil accumulation in a dose-dependent manner.[23] However, in their study preinjections of sites with AD did not inhibit neutrophil accumulation.

The direct toxicity of inhibitors of protein synthesis on neutrophils cannot account for the suppressed emigration, since their injection 60 or 90 min after the injection of cytokines or LPS did not have a significant effect. At this time emigration was maximal, however the majority of local RNA transcription and translation may have occurred earlier. Furthermore, protein synthesis inhibitors had no effect on neutrophil accumulation into lesions induced by the leukocyte chemoattractants FMLP, LTB_4 and C5a des arg. *In vitro* experiments, which have directly examined the effects of AD on neutrophils, did not show significant alterations in neutrophil function.[24]

Possibilities for decreased neutrophil access to the inflammatory site and consequently diminished accumula-

tion were also ruled out. Upon gaining access to the circulation, LPS can induce a prolonged neutropenia, with sequestration of neutrophils in various organs.[21] In our studies, relatively low doses of LPS were used and neutropenia was not observed. During the experimental period there was no evidence of vascular stasis, thrombosis or necrosis. Tissue necrosis was evident only after 24 hours in sites injected with AD and probably is the result of an irreversible block in RNA transcription.

Inhibitors of protein synthesis can decrease the production of prostaglandins, possibly by inhibiting the synthesis of cyclooxygenase enzymes.[25,26] This mechanism is not a likely explanation for the presented observations, since the inhibition of cyclooxygenase enzymes and prostaglandin production by indomethacin or acetylsalicylic acid predominantly diminishes local blood flow but has minimal effects on neutrophil emigration.[27,28]

Protein synthesis may be required in at least two steps of neutrophil emigration: in the adherence of neutrophils to endothelial cells and in the local production of cytokine mediators. The adherence of neutrophils to endothelial cells appears to be an important initial step in emigration elicited by bacterial products such as LPS or by leukocyte chemoattractants. *In vivo* this is illustrated both in natural and in experimental settings: a hereditary neutrophil deficiency/defect in CD11/CD18[29] or by blocking CD18 with antibodies.[23,30,31] CD11/CD18 is a family of leukocyte cell surface glycoprotein heterodimers, which mediate their ability to adhere to various surfaces including endothelial cells.[32] In these situations neutrophils cannot effectively emigrate into sites of infection or inflammation and patients with this hereditary condition suffer from recurrent life threatening bacterial infections.[27] *In vitro* LPS, IL-1 and TNF can directly increase endothelial cell adhesiveness for neutrophils[12–15] by a process at least in part mediated by inducible endothelial cell surface proteins.[16–18] Our results are consistent with the notion that this mechanism is important in the regulation of neutrophil emigration in response to LPS, IL-1 and TNF, however we cannot exclude the contribution of certain inflammatory cytokine mediators or leukocyte chemoattractants such as interleukin-8 (IL-8), whose generation is also dependent on protein synthesis.

In sites of inflammation IL-1 can be produced by monocytes/macrophages,[33] smooth muscle cells,[34] or endothelial cells,[35–37] and TNF by monocytes/macrophages.[38] Cytokines may amplify endothelial cell activation, or if the initial stimulus cannot directly stimulate endothelial cells, they may be the sole mediators of neutrophil emigration. The latter situation may occur with low doses of LPS,[6] since in culture relatively large concentrations of LPS (ng-ug/ml) are required to activate endothelial cells, whereas neutrophil accumulation can be detected *in vivo* when 1000 times lower concentrations are injected.

IL-8 (neutrophil-activating factor, monocyte-derived neutrophil chemotactic factor) is a potent inducer of neutrophil emigration in the rabbit and is not inhibited by AD.[39] This result and the reported results that inhibitors of protein synthesis have no effect on neutrophil emigration induced by leukocyte chemoattractants, do not imply that these inflammatory stimuli induce a protein synthesis-independent neutrophil emigration by diffusing into the vascular lumen and forming a chemotactic gradient. Desensitization or tachyphylaxis experiments (7.8) and the presented data with TNF (which is also a neutrophil chemoattractant) do not support this hypothesis. The mechanism of leukocyte chemoattractant-induced neutrophil emigration remains unknown. It is not known whether other mediators are produced locally, whether the leukocyte chemoattractants can directly stimulate endothelial cells to become hyperadhesive for neutrophils by a protein synthesis-independent mechanism, or whether they can somehow activate neutrophils in the blood.

References

1. Issekutz AC, Movat HZ: Quantitation of neutrophil infiltration *in vivo*. Immunol. Letters 1979, 1:27–30
2. Issekutz AC, Movat HZ: The *in vivo* quantitation and kinetics of rabbit neutrophil leukocyte accumulation in the skin, in response to chemotactic agents and *Escherichia coli*. Lab Invest 1980, 42:310–317
3. Kopaniak MM, Issekutz AC, Movat HZ: Kinetics of acute inflammation induced by *E. coli* in rabbits: Quantitation of blood flow, enhanced vascular permeability, hemorrhage and leukocyte accumulation. Am J Pathol 1980, 98:485–498
4. Colditz IG, Movat HZ: Kinetics of neutrophil accumulation in acute inflammatory lesions induced by chemotaxins and chemotaxinigens. J Immunol 1984, 133:2169–2173
5. Cybulsky MI, Colditz IG, Movat HZ: The role of interleukin-1 in neutrophil leukocyte emigration induced by endotoxin. Am J Pathol 1986, 124:367–372
6. Cybulsky MI, McComb DJ, Movat HZ: Neutrophil leukocyte emigration induced by endotoxin. Mediator roles of interleukin 1 and tumor necrosis factor alpha. J Immunol 1988, 140: 3144–3149
7. Colditz IG, Movat HZ: Chemotactic factor-specific desensitization of skin to infiltration of PMN-leukocytes. Immunol Letters 1984, 8:83–87
8. Colditz IG, Movat HZ: Desensitization of acute inflammatory lesions to chemotaxins and endotoxin. J Immunol 1984, 133: 2163–2168
9. Colditz IG: Kinetics of tachyphylaxis to mediators of acute inflammation. Immunol 1985, 55:149–156
10. Metchnikoff E: Lectures on the Comparative Pathology of Inflammation. Kegan, Paul, Trench, Truber and Co, 1893; London. 224pp (republished by Dover Publications, New York, 1968, 218pp)

11. Clark ER, Clark EL: Observations on changes in blood vascular endothelium in the living animal. Am J Anat 1935, 57: 385–438
12. Bevilacqua MP, Pober JS, Wheeler MS, Cotran RS, Gimbrone Jr. MA: Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes and related leukocyte cell lines. J Clin Invest 1985, 76:2003–2011
13. Dunn CJ, Fleming WE: The role of interleukin-1 in the inflammatory response with particular reference to endothelial cell-leukocyte adhesion. In *The Physiologic, Metabolic, and Immunologic Actions of Interleukin-1*. Kluger MJ, Openheim JJ, Powanda MC eds, 1985; Alan R. Liss, New York, pp 45–54
14. Gamble RJ, Harlan JM, Klebanoff SJ, Lopez AF, Vadas MA: Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. Proc Natl Acad Sci USA 1985, 82:8667–8761
15. Schleimer RP, Rutledge BK: Cultured human vascular endothelial cells acquire adhesiveness for neutrophils after stimulation with interleukin 1, endotoxin and tumor-promoting phorbol diesters. J Immunol 1986, 136:649–654
16. Pober JS, Bevilacqua MP, Mendrick DL, Lapierre LA, Fiers W, Gimbrone Jr MA: Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J Immunol 1986, 136:1680–1687
17. Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone Jr MA: Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci USA 1987, 84: 9238–9242
18. Smith CW, Rothlein R, Hughes BJ, Mariscalco MM, Schmalstieg FC, Anderson DC: Recognition of an endothelial determinant for CD 18-dependent human neutrophil adherence and transendothelial migration. J Clin Invest 1988, 82:1746–1756
19. McComb DJ, Cybulsky MI, Movat HZ: PMN emigration: protein synthesis dependent and independent mechanisms. Fed Proc 1987, 46:1390 (Abst.)
20. Cybulsky MI, Cybulsky IJ, Movat HZ: Neutropenic responses to intradermal injections of *E coli*. Effects on the kinetics of polymorphonuclear leukocyte emigration. Am J Pathol 1986, 124:1–9
21. Cybulsky MI, Movat HZ: Experimental pneumonia in rabbits: polymorphonuclear leukocyte margination and sequestration in rabbit lungs and quantitation and kinetics of ^{51}Cr-labeled polymorphonuclear leukocytes in *E. coli*-induced lung lesions. Exp Lung Res 1982, 4:47–66
22. Issekutz AC, Movat KW, Movat HZ: Enhanced vascular permeability and hemorrhage-inducing activity of zymosan-activated plasma. Clin Exp Immunol 1980, 41:505–511
23. Rampart M, Williams TJ: Evidence that neutrophil accumulation induced by interleukin-1 requires both local protein biosynthesis and neutrophil CD18 antigen expression *in vivo*. Br J Pharmacol 1988, 94:1143–1148
24. Cairo MS, Mallett C, Vande Ven C, Kempert P, Bennets GA, Katz J: Impaired *in vitro* polymorphonuclear function secondary to the chemotherapeutic effects of vincristine, adriamycin, cyclophosphamide and actinomycin D. J Clin Oncol 1986, 4:798–804
25. Bernheim HA, Dinarello CA: Effect of protein synthesis inhibitors on leukocytic pyrogen-induced *in vitro* hypothalamic prostaglandin production. Yale J Biol Med 1985, 58:179–187
26. Fagan JM, Goldberg AL: Inhibitors of protein and RNA synthesis cause a rapid block in prostaglandin production at the prostaglandin synthase step. Proc Natl Acad Sci USA 1986, 83:2771–2775
27. Issekutz AC, Bhimji S: The effect of nonsteroidal anti-inflammatory agents on *E. coli*-induced inflammation. Immunopharm 1982, 4:11–22
28. Issekutz AC, Bhimji S: Effect of nonsteroidal anti-inflammatory agents on immune complex- and chemotactic factor-induced inflammation. Immunopharm 1982, 4:253–266
29. Dana N, Arnaout MA: Leukocyte adhesion molecules (CD11/18) deficiency. In Bailliere's Clinical Immunology and Allergy, vol 2; Kazatchine M, ed. p. 453
30. Arfors KE, Lundberg C, Lindbom L, Lundberg K, Beatty PG, Harlan JM: A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage *in vivo*. Blood 1987, 69:338–340
31. Price TH, Beatty PG, Corpuz SR: *In vivo* inhibition of neutrophil function in the rabbit using monoclonal antibody to CD18. J Immunol 1987, 139:4174–4177
32. Pohlman TH, Stanness KA, Beatty PG, Ochs HD, Harlan JM: An endothelial cell surface factor(s) induced *in vitro* by lipopolysaccharide, interleukin 1 and tumor necrosis factor-alpha increases neutrophil adherence by a CDw18-dependent mechanism. J Immunol 1986, 136:4548–4553
33. Dinarello CA: Interleukin 1. Rev Infect Dis 1984, 6:51–95
34. Libby P, Ordovas JM, Birinyi LK, Auger KR, Dinarello CA: Inducible interleukin-1 expression in human vascular smooth muscle cells. J Clin Invest 1986, 78:1432–1438
35. Nawroth PP, Bank I, Handley D, Cassimeris J, Chess L, Stern D: Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. J Exp Med 1986, 163:1363–1375
36. Libby P, Ordovas JM, Auger KR, Robbins AH, Birinyi LK, Dinarello CA: Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells. Am J Pathol 1986, 124:179–185
37. Miossec P, Cavender D, Ziff M: Production of interleukin 1 by human endothelial cells. J Immunol 1986, 136:2486–2491
38. Beutler B, Cerami A: Cachectin. More than a tumor necrosis factor. N Engl J Med 1987, 3156:379–385
39. Colditz IG, Zwahlen R, Dewald B, Baggiolini M: *In vivo* inflammatory activity of neutrophil-activating factor, a novel chemotactic peptide derived from human monocytes. Am J Pathol 1989; 134:755–760