American Journal of Pathology, Vol. 139, No. 4, October 1991

Effects of All-trans Retinoic Acid on Neutrophil-mediated Endothelial Cell Injury *In Vitro* and Immune Complex Injury in Rats

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All-trans retinoic acid (RA) has beneficial effects when used in a variety of inflammatory skin conditions. In this study, the authors found that RA inhibited superoxide anion production and proteolytic enzyme release by human and rat neutrophils. Concomitantly, the authors found that RA-treated neutrophils were less able than untreated neutrophils to injure endothelial cells in culture even though the adhesion of the RA-treated neutrophils to endothelial cell monolayers was not diminished. Inhibition of cytotoxicity occurred over the same range of concentrations that inhibited oxygen radical formation and protease release. In additional studies, it was observed that pretreatment of endothelial cells with RA-induced resistance to subsequent injury by activated neutrophils. Finally, in vivo ***studies showed that pretreatment of rats for 3 days with RA (1–10 mg/day, IP) reduced the degree of injury in the lungs and skin sites after treatment with bovine serum albumin and antibodies to bovine serum albumin in the reverse-passive Arthus reaction. Thus, RA can modulate neutrophil-mediated endothelial cell injury by an effect on both the neutrophils and their target cells. Together, these effects may underlie the reduction in immune complex-mediated injury seen in experimental animals. The beneficial effects that retinoids have in a variety of inflammatory skin diseases may likewise be a reflection of their effects on the physiology of both neutrophils and endothelial cells. (Am J Pathol 1991, 139:901–909)***

Retinoids have beneficial effects when used in a variety of inflammatory conditions of the skin, including severe acne, psoriasis, and hidradenitis suppurativa.[1–5] The disappearance of neutrophils from these lesions has been observed after retinoid treatment in conjunction with therapeutic effects.[6] Since neutrophils play a central role in the acute inflammatory response, it has been suggested that the inhibition of neutrophil function may contribute to the beneficial effects of retinoids on inflammatory skin disease. In support of this possibility, *in vitro* studies have demonstrated that certain retinoids including all-trans retinoic acid (RA) and 13-cis retinoic acid inhibit chemotactic responses, superoxide anion (O_2-) production, and lysozomal enzyme release by neutrophils.[7–14] How retinoids exert their influence on neutrophils is not fully understood. At high concentrations, retinoids have detergentlike effects[15] and the inhibition of neutrophil function may be a manifestation of this. In support of this, at sublytic concentrations, detergentlike molecules stimulate the oxidative burst in neutrophils.[16] Similar effects have been observed with retinoids.[17] Regardless of the mechanism, the fact that retinoids can inhibit neutrophil function may explain, in part, their anti-inflammatory effects. The present studies were carried out to further characterize the effects of RA on the acute inflammatory response. The observations described in this report indicate that retinoic acid can influence the behavior of both the effector cells (neutrophils) and one of their major targets (endothelial cells). Together, these effects could contribute to the beneficial effects that retinoids have in a variety of inflammatory skin diseases.

Materials and Methods

Endothelial Cells

Rat pulmonary artery endothelial cells were isolated from the pulmonary vasculature by perfusion of microcarrier

Supported in part by grant HL42607 from the USPHS, by grant IM432 from the American Cancer Society, and by a grant from The R.W. Johnson Pharmaceutical Research Institute.

Accepted for publication June 10, 1991.

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beads into the vessels and subsequent retrieval of the beads with endothelial cells attached by retrograde perfusion.[18] On isolation, the cells exhibited the typical cobblestone morphology of endothelial cells. They were positive for factor VIII by immunofluorescence, bound acetylated low-density lipoprotein, and had high levels of angiotensin-converting enzyme (ACE) as measured with the synthetic substrate ^{3}H-Benzoyl-phe-ala-pro.[19] The cells were maintained in monolayer culture and utilized through passage 30. The culture medium consisted of minimal essential medium of Eagle with Earle's salts supplemented with nonessential amino acids, 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. The cells were passaged by trypsinization as required. Growth was at 37°C and 5% CO_2. Stocks were kept frozen in liquid N_2. Throughout the course of the study, the endothelial cells maintained their cobblestone morphology and levels of ACE activity.

Neutrophils

Human neutrophils were isolated from peripheral blood using a slight modification of Boyum's method as described previously.[20] The cells were finally suspended in Hank's balanced salt solution (HBSS) containing 0.02% bovine serum albumin (BSA) (HBSS-BSA) and kept on ice until use. Rat neutrophils were obtained by peritoneal lavage 3 to 4 hours after glycogen induction.[21]

Reagents

RA was obtained from Ortho Pharmaceutical Co. (Raritan, NJ). Stock solutions were prepared in dimethyl sulfoxide (DMSO) at a concentration of 20 mg/ml and stored frozen, protected from light. Working solutions were prepared in the appropriate culture medium at the time of use. When cells were treated with RA they were protected from light during the incubation period. The final concentration of dimethyl sulfoxide in the culture medium ranged from 0.02 to 0.10%. These concentrations of DMSO had no detectable effect. For *in vivo* use, RA was prepared in DMSO at a concentration of 100 mg/ml. At the time of injection, the RA was diluted in serumfree culture medium. Thus, at the highest concentration of RA, rats received 100 μl of DMSO. Control animals treated with this amount of DMSO were indistinguishable from untreated positive control animals. Phorbol 12-myristate 13-acetate (PMA), N-formyl-methionyl-leucyl-phenylalanine (FMLP), cytochalasin B, xanthine, xanthine oxidase, and N-succinyl-l-alanyl-l-alanyl-l-alanyl-para-nitroanilide (suc-ala-ala-ala-pNA) were obtained from Sigma Chemical Co. (St. Louis, MO).

Measurement of Superoxide Anion (O_2-) Production

O_2- generation was measured as the superoxide dismutase-inhibitable reduction of ferricytochrome C according to the method of Babior, Kipnes, and Curnutte.[22] The reaction mixture consisted of 2×10^6 unstimulated or stimulated neutrophils and 80 μm ferricytochrome C (obtained from Sigma Chemical Company) in 0.9 ml of HBSS. To one set of duplicate tubes was added 0.1 ml of HBSS whereas 85 units of superoxide dismutase (SOD) was added to the other set. The tubes were incubated for 60 minutes at 37°C after which the total volume was brought to 1.8 ml. The tubes were centrifuged and the absorbance of the supernatant fluids at 550 nm was determined. The difference in absorbance between the presence and absence of SOD was determined and the amount of ferricytochrome C reduced was calculated based on an extinction coefficient of 18.5 cm^{-1} mM^{-1} for ferricytochrome C.

Measurement of Proteolytic Enzyme Activity

Hydrolysis of hemoglobin by unstimulated and stimulated neutrophils was used as a measure of proteolytic enzyme activity.[23] Bovine hemoglobin was prepared as a 5 mg/ml solution in HBSS and 0.5 ml of the hemoglobin solution was incubated with 2×10^6 neutrophils in 0.1 ml for 4 hours at 37°C. After incubation, 0.25 ml of 40% trichloroacetic acid was added to precipitate the protein. The tubes were left at room temperature for an additional 15 minutes and then were centrifuged at 2000 rpm for 20 minutes. Following this, 0.5 ml of the supernatant fluid was removed from each tube and the amount of proteolytic fragments remaining in solution was determined by the Lowry method,[24] in comparison with a BSA standard.

In addition to hemoglobin hydrolysis, we also examined the cleavage of the synthetic substrate suc-ala-ala-ala-pNA as a measure of elastolytic activity. The assay was carried out as described in our past report.[21] Essentially, 2×10^6 unstimulated or stimulated neutrophils were incubated for 30 minutes in HBSS. After separation of the cells by centrifugation, 0.5 ml of supernatant fluid was incubated for 18 hours with 1 ml of suc-ala-ala-ala-pNA (1.25 mg/ml). The amount of pNA released from the peptide was determined by measuring the change in optical density at 405 nm.

Cytotoxicity Assay

Cytotoxicity was measured using a standard ^{51}Cr-release assay.[25] The endothelial cells were seeded into

wells of a 24-well culture dish at 1.0×10^5 cells per well in 1 ml of culture medium. Each well received 1 μCi of $Na^{51}CrO_4$ (New England Nuclear; Boston, MA). The cells then were incubated for 18 hours after which they were washed twice to remove unincorporated radioactivity and were ready to use. Suspensions of neutrophils in HBSS-BSA were added to duplicate wells to give the desired effector to target ratios in a final volume of 1.0 ml. The neutrophils were allowed to settle onto the endothelial cell monolayer for 30 minutes prior to the addition of the stimulating agent (50 nM PMA) which was added in a volume of 0.1 ml per well. After an additional incubation at 37°C for 4–6 hours, 0.9 ml of supernatant was removed from each well and centrifuged. The supernatant (0.5 ml) was aspirated and assayed in a γ-scintillation counter to determine ^{51}Cr release. Spontaneous release was obtained from wells receiving medium only and total release was obtained from wells receiving 0.2% Triton X-100. The spontaneous release never exceeded 20% of the total release and in most experiments ranged between 5 and 10% of the total release. The percentage of cytotoxicity was calculated by the following formula:

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental release} - \text{spontaneous release}}{\text{Total release} - \text{spontaneous release}}$$

Adhesion Assay

Endothelial cells were seeded into the wells of a 24-well culture dish as described for the cytotoxicity assay except that they were not ^{51}Cr-labeled. The cells were incubated for 18 hours after which they were washed twice and used. Suspension of neutrophils were added to duplicate wells at 5×10^5 cells per well and allowed to settle onto the endothelial cell monolayer for 30 minutes. PMA (50 nM) was added and the cells incubated for one hour. Non-attached neutrophils were then removed and the endothelial cell monolayers washed gently two times. The washes were added to the tubes containing the non-attached neutrophils and the entire sample counted. The percentage of attached cells was determined from this.

Animal Studies

Male pathogen free Long-Evans rats (250–300 grams) (Charles River, Portage, MI) were used in these studies. To produce lung injury, a tracheostomy incision was surgically performed and a fine Teflon catheter was inserted into the distal trachea under Ketamine anesthesia. During inspiration, 100 μg of affinity purified rabbit antibovine serum albumin IgG was instilled into the airways in a total volume of 0.25 ml. The catheter was then withdrawn and the incision closed with silk sutures. Immediately following closure of the incision, the animals were injected intravenously with 10 mg of crystalline BSA. Quantitation of lung injury was accomplished by the intravenous injection of 1 μg of rat ^{125}I-IgG along with the BSA. Four hours later, the animals were sacrificed. The pulmonary vasculature was perfused with 10 ml of saline, with drainage of the perfusate being accomplished by aortic transection. Residual lung radioactivity was assessed by counting radioactivity in a gamma scintillation counter and a lung: blood ratio of lung permeability was obtained. To obtain morphologic evidence of injury, lungs from animals treated in the same manner (but without ^{125}I-IgG) were removed, inflated with formaldehyde and embedded in paraffin. The embedded tissues were cut, stained with hematoxylin and eosin and examined under light microscopy. The pathophysiological basis of the lung injury has been previously characterized in this model.[26]

Injury to the skin was accomplished in a similar manner except that the antibody was injected into shaved skin sites rather than instilled into the airways. Normally, 100 μg was injected per skin site in a total volume of 50 μl. This model of immune-complex injury has also been described in a previous report.[27]

Results

Effects of RA on O_2- Production and Proteolytic Enzyme Release by PMA-stimulated Human and Rat Neutrophils

In the first series of experiments, RA was examined for its ability to interfere with the functions of neutrophils that contribute to the inflammatory process. Consistent with previous findings of others,[7,8,10,11,13] we found that pretreatment of human neutrophils for 45 minutes with concentrations of RA ranging from 1–20 μg/ml interfered with generation of O_2- and with proteolytic enzyme release. Results demonstrating inhibitory effects of RA on O_2- generation are shown in Figure 1. Minimal inhibition was seen at 1 μg/ml and a 50% inhibition was achieved at approximately 10 μg/ml.

The same concentrations of RA that inhibited O_2- generation also inhibited proteolytic enzyme release from activated neutrophils. Data obtained using hemoglobin hydrolysis as a marker are shown in Figure 2A. When the elastase-specific substrate, suc-ala-ala-al-pNA, was used, inhibition was even greater (Figure 2B). Inhibition of oxygen radical production and proteolytic enzyme release by RA did not appear to be a reflection of nonspecific toxicity because when RA-treated neutrophils were stained with trypan blue after incubation in culture for 45

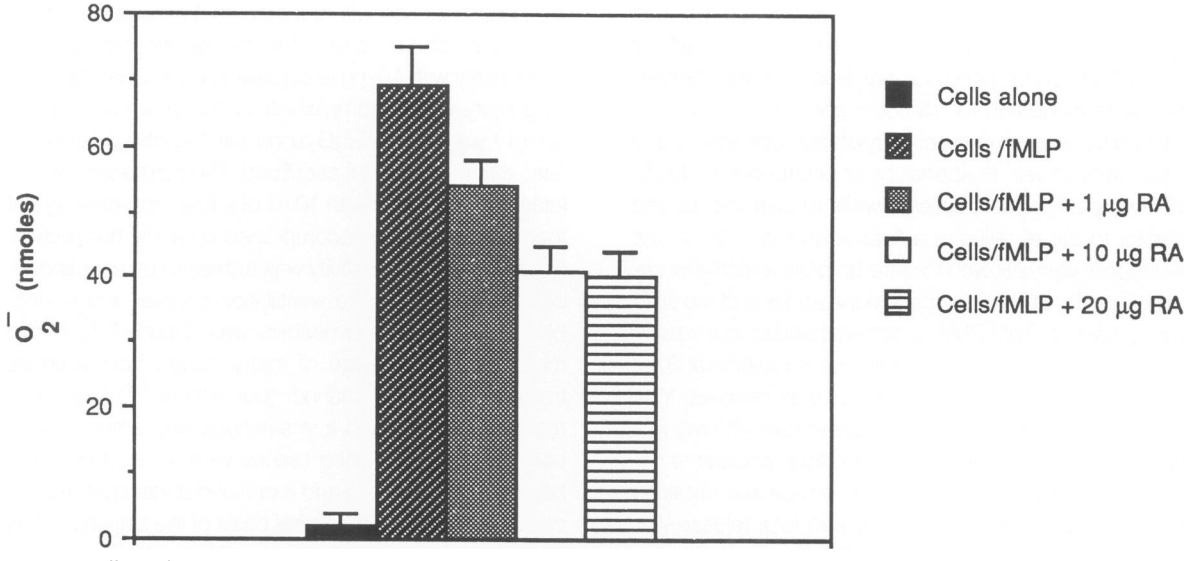

Figure 1. *Effects of RA on O_2-generation by human neutrophils. Neutrophils were treated for 45 minutes with the desired amount of RA prior to addition of the agonist. The agonist consisted of 1 × 1 0^{-6} M FMLP in the presence of cytochalasin B at a concentration of 1 μg/ml. Values shown represent average nmols of O_2-generated per 2 × 10^6 cells per hour and standard errors based on four independent experiments with duplicate samples per data point.*

minutes, there was no increase in the number of staining cells over that seen in untreated controls (not shown).

Rat neutrophils obtained from glycogen-induced peritoneal exudates were also examined for O_2- production and proteolytic enzyme release. Although these cells produced smaller amounts of O_2- and released smaller amounts of proteolytic enzymes than human cells on a per-cell basis, they responded to RA in a similar manner to the human neutrophils. A 50% inhibition of O_2- production was achieved at approximately 5 μg/ml (6 nmols vs. 13 nmol per 2 × 10^6 cells per 30 minutes). Likewise, 5 μg/ml of RA caused a 30% inhibition of proteolytic enzyme release whereas a 75% inhibition was achieved at 15 μg/ml.

The ability of RA to inhibit O_2- production was probably not due to a direct scavenging effect because when O_2- and hydrogen peroxide were generated using xanthine and xanthine oxidase, we saw no inhibition in the presence of 1 to 20 μg/ml of RA (not shown).

Effects of RA on Neutrophil Adhesion to and Killing of Endothelial Cells

We next examined RA for its ability to modulate neutrophil adhesion to monolayers of rat pulmonary artery endothelial cells. For this, 5 × 10^5 human neutrophils were treated with RA (1–20 μg/ml) as described earlier and then washed and added to monolayers of the endothelial cells. PMA (5 nM) was added to activate the neutrophils and the percentage of neutrophils adherent to the endothelial monolayers determined 1 hour later. As seen in Table 1, RA had no effect on the ability of the neutrophils to attach to endothelial cells.

In previous studies we demonstrated that PMA-activated human neutrophils were capable of killing endothelial cells in a process that depended primarily on the generation of oxygen radicals but also involved neutrophil proteases.[25,28–32] Since RA inhibited both oxygen radical formation and protease activity, it seemed reasonable to suggest that the same treatment would reduce injury to the endothelium. To test this, human neutrophils were treated for 45 minutes with 1 to 20 μg/ml of RA and then washed. The treated neutrophils and untreated control cells were then examined for ability to injure endothelial cells in a 4-hour cytotoxicity assay. It was found that the RA-treated neutrophils were less cytotoxic than untreated control neutrophils (Table 2). Inhibition of cytotoxicity occurred over the same range of RA concentrations that inhibited oxygen radical formation and enzyme release (1–20 μg/ml). No inhibition of cytotoxicity was observed when the neutrophils were treated with a lower RA concentration (0.1 μg/ml) (not shown).

Effects of RA on Endothelial Cell Sensitivity to Killing by Neutrophils

Additional experiments were carried out in which the endothelial cells, themselves, were treated for 18 hours with RA and then washed and examined for sensitivity to killing by PMA-activated neutrophils. For these experiments, the endothelial cells were plated in wells of a 24-well dish and treated with RA (1–20 μg/ml) in complete culture

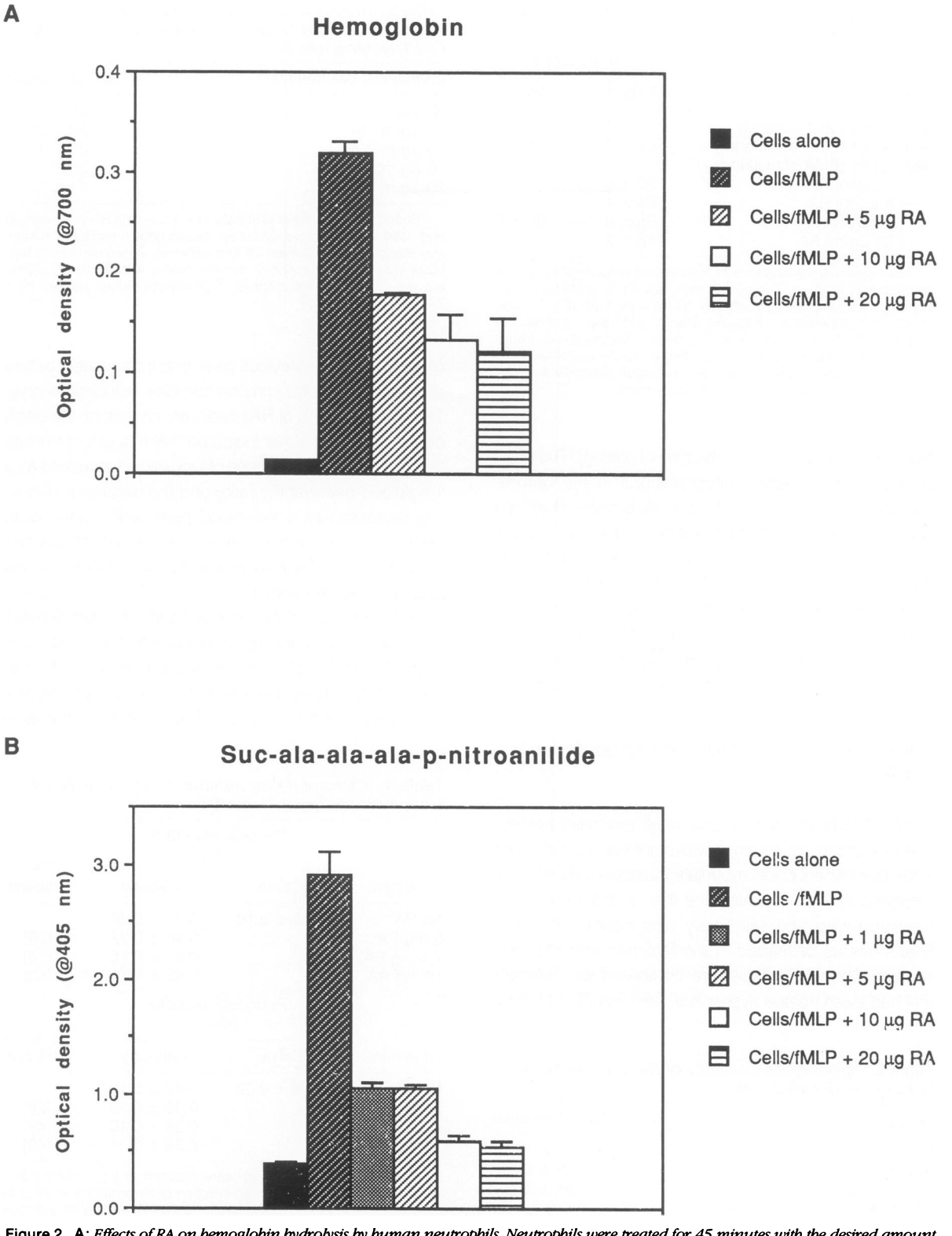

Figure 2. **A:** *Effects of RA on hemoglobin hydrolysis by human neutrophils. Neutrophils were treated for 45 minutes with the desired amount of RA prior to addition of the agonist. The agonist consisted of 1 × 10^{-6} M FMLP in the presence of cytochalasin B at a concentration of 1 μg/ml. Values shown represent means and standard errors based on three independent experiments with duplicate samples per data point.* **B:** *Effects of RA on suc-ala-ala-ala-pNA hydrolysis by human neutrophils. Neutrophils were exposed to RA for 45 minutes following which they were stimulated for an additional 30 minutes with 1 × 10^{-6} M FMLP in the presence of cytochalasin B (1 μg/ml). The supernatant fluid was then collected and incubated with the substrate for 18 hours. Values shown represent average optical density change ± the difference average values and individual values based on duplicate samples per data point in a single experiment. The experiment was repeated three times with similar results.*

Table 1. *Effects of RA on Human Neutrophil Adhesion To Endothelial Cells*

Group	% Attached	
	Exp. 1	Exp. 2
Neutrophils (unstimulated)	5 ± 1	9 ± 1
Neutrophils (PMA-stimulated)	67 ± 2	94 ± 1
Neutrophils (PMA-stimulated)		
+1 μg/ml RA	70 ± 5	—
+5 μg/ml RA	65 ± 4	—
+10 μg/ml RA	68 ± 8	92 ± 2
+20 μg/ml RA	66 ± 2	—

Neutrophils were treated with the desired amount of RA for 45 minutes and washed. They were then added to endothelial cell monolayers and allowed to settle for 30 minutes. PMA (50 nM) was added and adherence measured after 1 additional hour as described in the Materials and Methods section. Values shown represent the percentage of neutrophils attached to the endothelial cell monolayers ± the difference between averages and individual values based on duplicate samples per data point.

medium along with ^{51}Cr (at the time of plating). The presence of RA at these concentrations in the serum-containing culture medium had no detectable effects on endothelial cell viability, proliferation or ability to incorporate ^{51}Cr. The cytotoxicity assay was carried out 1 day later in the normal manner. As seen in Table 3, RA reduced endothelial cell sensitivity to neutrophil-mediated killing. It thus appears that RA can interfere with neutrophil-mediated injury to vascular endothelial cells by an effect on the target cells as well as on the neutrophils.

Effects of RA on Immune-complex Injury in the Rat

Since RA was able to modulate neutrophil-mediated endothelial cell injury *in vitro*, it was of interest to determine if the same agent could modulate the acute inflammatory response *in vivo*. To determine this, immune-complex–mediated lung and skin injury was induced in Long-Evans rats as described in the Materials and Methods section. Control animals were compared with animals that had been treated systemically with RA (5–10 mg/rat

Table 2. *Effects of RA on Ability of Human Neutrophils to Injure Endothelial Cells*

Group	% ^{51}Cr-release
Buffer alone	8 ± 1
Neutrophils (Unstimulated)	8 ± 0
Neutrophils (PMA-stimulated)	31 ± 3
Neutrophils (PMA-stimulated)	
+1 μg/ml RA	27 ± 3
+10 μg/ml RA	22 ± 2
+20 μg/ml RA	16 ± 2

Neutrophils were treated for 45 minutes with the desired amount of RA and then washed and used in the cytotoxicity assay. Values represent means and standard errors based on four separate experiments with triplicate samples per data point.

Table 3. *Increased Sensitivity of Endothelial Cells to Injury by Activated Neutrophils Following Endothelial Cell Treatment with RA*

Endothelial cell treatment[1]	% ^{51}Cr-release
No RA	40 ± 1
1 μg/ml RA	28 ± 2
5 μg/ml RA	25 ± 1
10 μg/ml RA	25 ± 3
20 μg/ml RA	21 ± 2

Endothelial cells were pretreated for 1 day with RA and washed and used in the cytotoxicity assay. Values shown represent means and standard errors based on four separate experiments with triplicate samples per data point. In cells treated with RA (0–20 μg/ml) but not exposed to neutrophils, ^{51}Cr-release values ranged from 7–10%.

given IP on the 2 previous days and immediately before establishment of the immune complex-induced lesions). The concentrations of RA used were chosen on the basis of previous studies that indicated that rats would tolerate this treatment.[33] The degree of lung injury (assessed as a lung/blood permeability ratio) and the degree of skin injury (assessed as a skin/blood permeability ratio) were both significantly decreased in the animals that had been treated with RA (Table 4). Histologic examination of lungs and skin sites are shown in Figure 3. The lung and skin inflammatory sites in the positive control animals showed a marked influx of neutrophils along with edema and cell injury (Figure 3a, c). In the RA-treated animals, the degree of tissue injury was less but the numbers of neutrophils present at the sites appeared to be the same as in

Table 4. *Effects of RA on Immune Complex-mediated Lung and Skin Injury in the Rat*

Treatment	Permeability index (lung)		% Inhibition
	Saline	Antibody	
No RA	0.30 ± 0.10	0.71 ± 0.37	
5 mg RA		0.48 ± 0.22	(56)
7.5 mg RA		0.41 ± 0.15	(74)
10 mg RA		0.45 ± 0.16	(63)
Treatment	Permeability index (skin)		% Inhibition
	Saline	Antibody	
No RA	0.04 ± 0.02	0.47 ± 0.12	
5 mg RA		0.33 ± 0.15	(28)
7.5 mg RA		0.26 ± 0.10	(49)
10 mg RA		0.26 ± 0.11	(49)

Skin injury and lung injury were induced in fully anesthetized Long-Evans rats by intradermal injection or intratracheal instillation of rabbit antibody to bovine serum albumin followed by intravenous injection of bovine serum albumin and rat ^{125}I-IgG. Treated animals had been given intraperitoneal injections of RA once a day for the 2 previous days and a third intraperitoneal injection of RA immediately before treatment with bovine serum albumin and antibody to bovine serum albumin. Lung values represent averages ± standard deviations based on groups of rats ranging from 9–11 rats/group. Skin values represent averages ± standard deviations based on 23–42 individual skin sites on 7–11 individual rats/group.

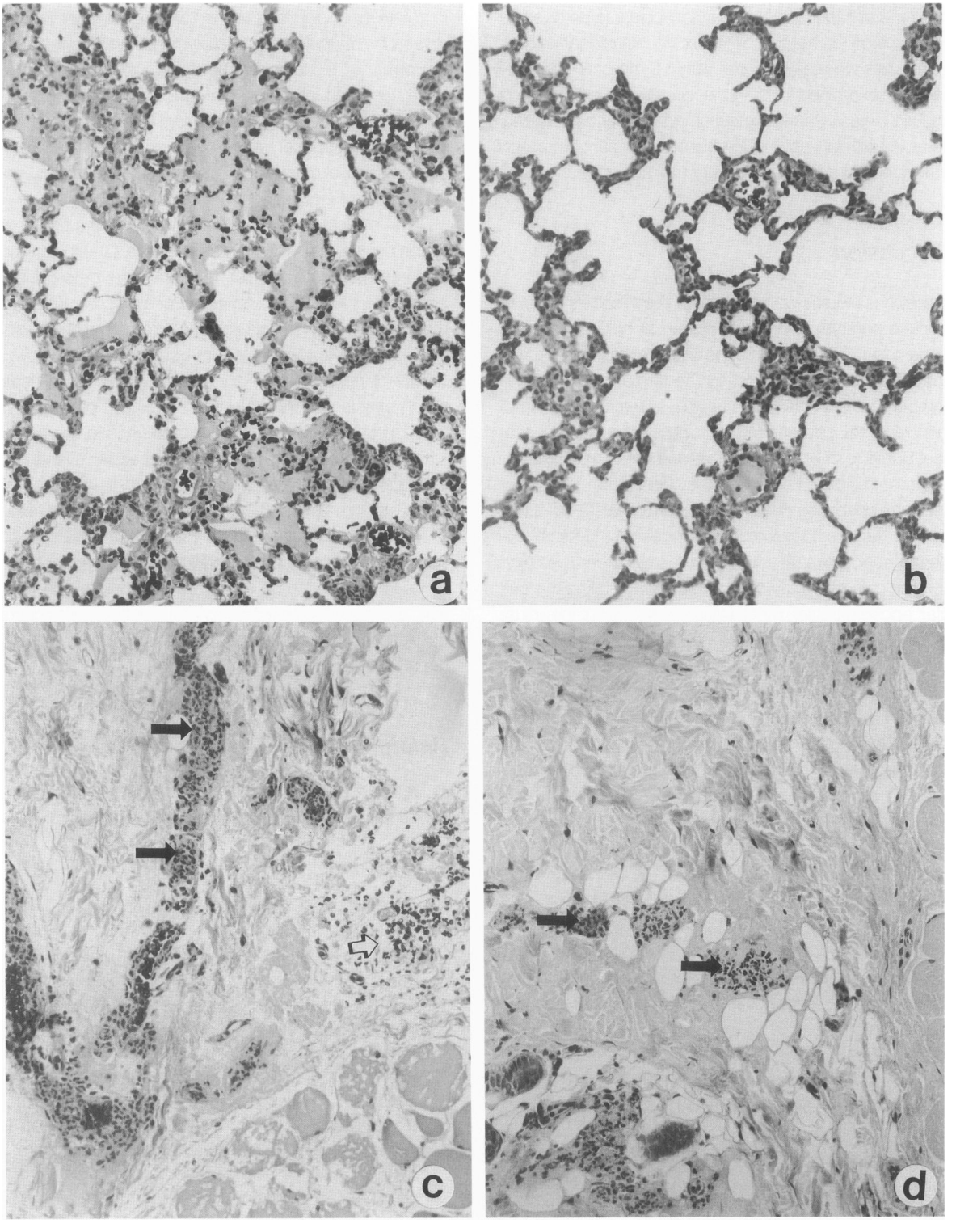

Figure 3. *Histologic studies of skin and lung in positive control and RA-treated, immune complex-injured rats. In the lungs of animals injured with IgG immune complexes there is neutrophilic infiltration along with intraalveolar hemorrhage and edema* (**a**). *By comparison, in rats given RA in addition to the IgG immune complexes there are also neutrophils but there is a marked suppression of the lung injury as evidenced by diminished intraalveolar hemorrhage and edema* (**b**). *In the dermal vasculitis model the degree of neutrophilic infiltration into the dermal vessels appears equal in the IgG-control* (**c**) *and IgG plus RA-treated rats* (**d**) *(solid arrows). However, as in the lung, the RA-treated rats do not show the extravascular edema, hemorrhage and necrosis (open arrows) that is present in the positive control rats, (H&E, ×85).*

the positive controls (Figure 3b, d). Thus, the anti-inflammatory effect of RA does not appear to be due to a suppression of neutrophil influx. In additional experiments, rats were also treated with 1.0 mg of RA per day and then examined for immune- complex–mediated lung and skin injury. Partial protection against lung injury was obtained with this dose of RA but no significant protection was obtained against skin injury (not shown).

Discussion

In previous studies we have established models of acute immune complex–mediated inflammatory disease in the lungs and skin of rats.[26,27] The disease in both skin sites and lungs is characterized by the formation of immune complexes within the vascular wall, activation of complement and recruitment of neutrophils. The presence of neutrophils is critical to the development of the tissue injury in both sites. There is clear evidence that lung injury depends on the formation of oxygen radicals[26] but a role for other factors has also been suggested.[34] Indirect evidence suggests that in the skin, factors other than oxygen radicals may play critical roles.[27] To define the cellular and molecular mechanisms underlying the injury process, we have examined neutrophil-mediated injury to endothelial cells in culture. Using bovine and rat pulmonary artery endothelial cells as targets, our studies have shown that neutrophil-mediated injury is oxygen radical-dependent and involves generation of the hydroxyl radical by ferrous iron reduction of hydrogen peroxide in a Fenton-type reaction.[25,28–31] These studies have shown further that the generation of the hydroxyl radical probably occurs within the endothelial cells utilizing iron from the endothelial cells[25,28] as well as oxidants generated within the target cells.[29–31] Further, our *in vitro* studies have suggested that although oxygen radicals are critical for injury, other neutrophil products including proteolytic enzymes, cationic proteins and membrane active agents act synergistically with the oxidants to maximize injury.[32,35]

The present studies indicate that RA is capable of modulating the immune complex-induced injury in both the skin and lungs *in vivo* and is also capable of interfering with neutrophil-mediated injury to the endothelium *in vitro.* How protection from acute inflammatory injury is brought about by RA is not known. *In vitro* studies suggest that RA may act at the level of both the effector cell population (neutrophils) and the target cells to bring about a reduction in inflammatory injury. We conclude the reduction in both lung and skin Arthus type reactions in the RA-treated, immune complex-injured rats is a result of effects on both the neutrophils and one of their major targets. One can speculate that the same mechanisms underlie the ability of retinoids to provide effective therapy in a variety of skin conditions that are, at least in part, reflections of acute inflammatory responses. In the past, the ability of retinoids to suppress conditions such as acne has been largely attributed to their inhibition of follicular keratinization.[1–5] The present data do not conflict with these past observations but suggest that leukocytes and endothelial cells as well as the cells of the epidermis are targets of retinoid action.

The molecular mechanisms underlying the effects of RA on neutrophil function and endothelial function are not known. As indicated earlier, retinoids have detergentlike properties[15] and effects observed at high concentrations may be the result of this activity. Alternatively, retinoids influence the expression of numerous gene products in a variety of cells.[36] It is likely to be difficult to delineate which effects are critical to the modulation of neutrophil function. With regard to the endothelium, we are beginning to understand some of the intracellular events that regulate susceptibility to injury.[25,29–31,37] It will be possible to determine if one or more of these events is a target of RA. As our understanding of the basic mechanisms of neutrophil-mediated endothelial cell injury increases, this will aid our efforts to delineate how RA acts in inflammation. Likewise, as we learn more about the mechanisms of RA action, this could lead to a better understanding of the inflammatory process, in general.

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