

Neutrophil Function in Psoriasis: Effects of Retinoids

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The present investigation focused on the oxidative response of polymorphonuclear neutrophil leukocytes in psoriasis, in particular pustular psoriasis and how this response was affected by different retinoid compounds. In the active phase of pustular psoriasis, the neutrophil chemiluminescence response to the chemotactic peptide f-met-leu-phe and to phorbol myristate acetate was enhanced and correlated to the development of pustules, whereas cells from psoriasis vulgaris patients showed normal chemiluminescence response. Retinoids, particularly tretinoin (= retinoic acid) and isotretinoin caused a pronounced inhibition of the chemiluminescence response only in primed neutrophils in vivo and in vitro, whereas etretinate and the metabolite Ro 10-1670 were less inhibitory. Retinoic acid furthermore inhibited the Fc-mediated phagocytosis, but did not affect C3bi-mediated phagocytosis. These data suggest that the antiinflammatory effect of retinoids may operate by affecting neutrophil activation and function. (Received March 5, 1987.)

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In psoriatic patients there is an accumulation of granulocytes, in particular polymorphonuclear neutrophil leukocytes (neutrophils), in lesional skin. In pustular psoriasis the abscess formation is pronounced and results in the formation of apparently sterile pustules. How these pustules are formed is unclear, but various chemotactic factors have been isolated from psoriatic skin of both vulgaris and pustular type (1). However, contradictory reports have been published on the role of neutrophils in the pathogenesis of psoriatic disease (2, 3, 4, 5, 6, 7). Most regimens used for the treatment of psoriasis do not directly aim at inhibiting the neutrophil response in the inflammatory lesion, although some reports show altered neutrophil function after treatment (8, 9, 10, 11). It is however, hard to overlook the role of these cells in psoriasis, particularly in pustular psoriasis.

Retinoid compounds have been successfully used in the treatment of various inflammatory diseases (12, 13) and regarding psoriasis the most beneficial effects have been obtained in the pustular forms. Therefore, it seems worthwhile to further elucidate the function of neutrophils in psoriasis as well as the effect of retinoids on neutrophil function.

The present investigation was prompted by our finding that there is a correlation between disease activity and chemiluminescence response of neutrophils in pustular psoriasis. The beneficial effects of retinoids in the treatment of pustular psoriasis may well be the result of effects on neutrophils, since it has been shown that retinoid compounds inhibit several cellular functions in neutrophils (14, 15, 16, 17). We have demonstrated that retinoic acid selectively affects the development of certain chemotactic receptors during cell differentiation (18). The present investigation focuses on the effect of various retinoid compounds on different cellular functions, primarily the oxidative response, of primed neutrophils.

MATERIAL AND METHODS

Patients

The investigation included 8 patients with chronic, stable plaque psoriasis, 5 with pustular psoriasis of the Zumbusch type, and non-psoriatic healthy volunteers. One patient with pustular psoriasis, a 52-year-old female, has had episodes of generalized pustulation at least once a year for more than 30 years. She has not accepted any treatment except baths and topical emollients, thus giving us the opportunity to investigate the spontaneous course of some of her pustular episodes. The white blood count was elevated in patients with pustular psoriasis as well as plaque psoriasis.

Cells

Blood was obtained by the use of EDTA-vacutainer tubes (Becton Dickinson Co, Orangeburg, N.Y.). The polymorphonuclear neutrophil leukocytes were separated according to the method of Böyum (19). Remaining erythrocytes were removed by hypotonic lysis and the neutrophils were washed twice in Krebs-Ringers' phosphate buffer supplemented with 10 mM glucose, pH 7.2 (KRG), and finally suspended to 1×10^7 cells per ml in the same buffer. Viability (>95%) was tested by using trypan blue exclusion.

Chemiluminescence measurement

This was done using a Luminometer 1250 (LKB Wallac, Stockholm, Sweden) kept at 22°C (20). Reaction samples were obtained by adding 0.4 ml KRG, 0.01 ml luminol (1 mg/ml) and 0.1 ml neutrophil suspension (10^7 /ml) to disposable 4 ml polypropylene tubes. The tubes were placed in the luminometer and allowed to equilibrate for two minutes. To activate the cells, 0.1 ml of the appropriate stimulus phorbol myristate acetate (PMA, 10^{-6} M), formylated methionyl-leucyl-phenyl-alanine (fMLP, 10^{-6} M), or serum-treated zymosan (STZ, 5 mg/ml) was added, the tubes stirred and the light emission recorded continuously for at least 30 min.

Myeloperoxidase-mediated iodination

This was carried out as previously described (6). The reaction mixture contained 10^6 cells, 30 nmol sodium iodide ($0.5 \mu\text{Ci } ^{125}\text{I}$), retinoids (25 μM) and KRG to a final volume of 0.5 ml. The cells were preincubated with 2×10^{-7} M fMLP or with buffer for 5 min and then stimulated with 10^{-6} M PMA for 20 min at 37°C.

The reaction was terminated by adding 0.1 ml 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ and 5 ml cold trichloroacetic acid (TCA). After centrifugation the precipitate was washed 3 times with cold TCA. The protein bound radioactivity was then detected in a gamma-scintillation counter.

Phagocytosis

The phagocytic uptake of IgG and C3bi-opsonized yeast particles (*Saccharomyces cerevisiae*) was assayed as previously described using the fluorescence quenching method (21).

Retinoids

The following retinoid compounds were used: retinoic acid, etretinate (Ro 10-9359), metabolite of etretinate (trimethylmethoxyphenyl retinoic acid Ro 10-1670), isotretinoin (13-cis-retinoic acid, RO 4-3780) and tretinoin (Ro 1-5488) (= retinoic acid). All retinoid compounds were dissolved in DMSO and stored protected from light. The final concentration of DMSO did not exceed 0.1%. The concentrations of retinoid compounds used in the experiments were calculated to be equivalent to serum levels obtained in clinical use (22). The retinoid compounds were kindly provided by Hoffman-LaRoche.

Reagents

PMA, fMLP, zymosan, and luminol were purchased from Sigma Chemical Co (St Louis, Mo).

RESULTS

Neutrophil function in pustular psoriasis

In the patients with pustular psoriasis neutrophil chemiluminescence was enhanced ($p < 0.01$) particularly during pustular episodes, when stimulated with either fMLP or PMA (Table I). The response to STZ was also enhanced (not shown by figure or table). Especially the response to fMLP is greatly enhanced. However, no such increase in chemiluminescence response was seen in patients with psoriasis vulgaris (Table I). In one

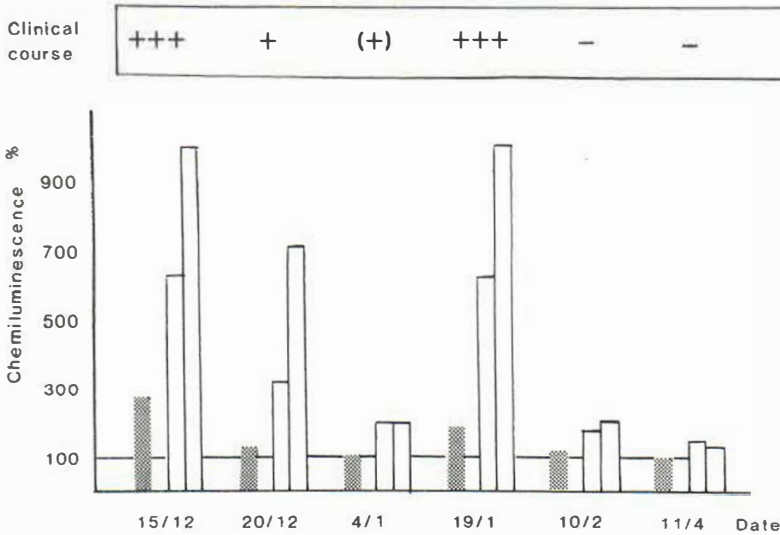


Fig. 1. Chemiluminescence (CL) pattern of granulocytes from an untreated patient with pustular psoriasis, correlated to clinical course of the disease and expressed as % CL of a control run in parallel. (■) PMA as stimulus, (□) fMLP as stimulus, with characteristic two-peak pattern in active disease.

patient with psoriasis vulgaris whose psoriasis changed into exudative form, with visible pustules in the plaques, we demonstrated an increase in chemoluminescence response at that time. One patient did not receive any antiinflammatory treatment or medication of any kind during the time period studied and her pustular bouts correlated well with enhanced oxidative response both to fMLP and PMA (Fig. 1).

In the primed, i.e. in vivo activated, cells from patients with active pustular psoriasis a two-peak pattern with a pronounced second peak was registered (Fig. 2A), measuring chemiluminescence, using fMLP as stimulus, while in the non-psoriatic control cells a pronounced first peak, but no, or a very discrete second one was found (Fig. 2B). The first peak reflects the extracellular generation and the second peak the intracellular generation of oxidative metabolites. After preincubation of the non-psoriatic control cells at room temperature for 60 min, the chemiluminescence response increased and a second peak developed in the cells (Fig. 2C).

Cells from pustular psoriasis patients did not show such increase of the response to fMLP after preincubation at room temperature. To evaluate whether the in vivo priming of the pustular psoriasis neutrophils was due to some soluble serum factor, we preincubated normal neutrophils with serum collected from a patient with an episode of pustular psoriasis. We could not in these cells mimic the response seen in the in vivo primed neutrophils of pustular psoriasis.

Table I. The fMLP and PMA-induced chemiluminescence response^a in neutrophils from pustular psoriasis (P.P.), pustular vulgaris (P.V.) and healthy controls

Stimulus	Neutrophils from			
	P.P.	P.P. without pustules	P.V.	Exudative P.V.
fMLP	504±79	203±32	102±5	200
PMA	178±28	123±25	105±6	420

^a Expressed as % of chemiluminescence response in control cells, stimulated with 10^{-7} M fMLP or PMA. Mean ± SEM.

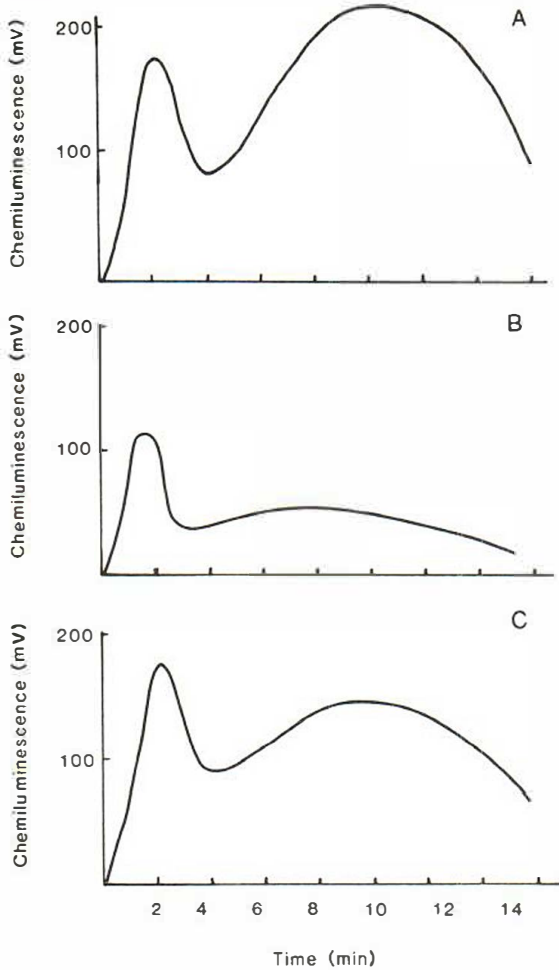


Fig. 2. Chemiluminescence response of granulocytes using fMLP (10^{-7} M) as stimulus. (A) Granulocytes from patients with pustular psoriasis. (B) Normal granulocytes. (C) Normal granulocytes preincubated for 60 min at 20°C .

Effect of retinoids on neutrophil function

To evaluate the potential antiinflammatory effect of retinoids on neutrophil function, different retinoid compounds (etretinate, tretinoin, isotretinoin and Ro 10-1670) were incubated with neutrophils. A dose-dependent inhibition of PMA- (Fig. 3), fMLP- (Fig. 4) and STZ- (not shown by figure) induced chemiluminescence response by retinoic acid, tretinoin (= retinoic acid) and isotretinoin could be demonstrated. The inhibitory effect of etretinate was rather weak, while the metabolite (Ro 10-1670) had no effect at the concentration tested (1–25 μM).

When scrutinizing the chemiluminescence response to fMLP, we find that the retinoids affect primarily the second peak, whereas the initial peak is only slightly affected (Fig. 5). The inhibitory effect of retinoids was thus seen in neutrophils primed *in vivo* from pustular psoriasis patients in pustular bouts and in primed control cells. To evaluate whether the retinoids affected the development of the second peak response, reflecting the intracellular generation of oxidative metabolites, we exposed neutrophils to retinoids (isotretinoin and tretinoin) for 60 min and then tested the chemiluminescence response to fMLP after washing off the drug. This exposure to retinoids did not affect the development of the second peak, if the drug was present only during preincubation. The drug had to be present

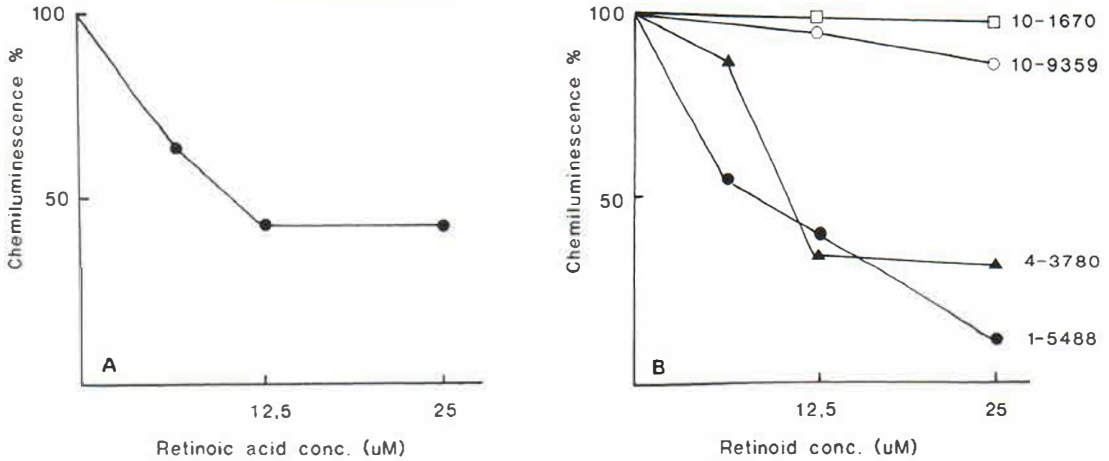


Fig. 3. Effect of (A) retinoic acid and (B) other retinoids, on chemiluminescence of activated granulocytes using PMA (10^{-7} M) as stimulus. CL is expressed as % of control cells exposed to 0.1% DMSO.

during the activation of the cells with fMLP in order to exert its inhibitory effect. When studying Fc-mediated phagocytosis, we detected no difference between pustular psoriasis, exsudative psoriasis vulgaris, and control cells (Table 11). C3bi-mediated phagocytosis was however significantly enhanced ($p < 0.01$) in psoriasis vulgaris neutrophils. In Fig. 6 it is shown that retinoic acid inhibited the IgG-mediated phagocytosis in a dose-dependent fashion, while the C3b-mediated phagocytosis was not affected. This applied to both control cells and pustular psoriasis cells exposed to retinoic acid *in vitro*.

In previous investigations we found normal myeloperoxidase-mediated iodination in pustular psoriasis, stimulated with yeast cells (6). This is also the case when PMA or fMLP is used as stimulus. When neutrophils are pretreated with fMLP and subsequently stimulated with PMA, the myeloperoxidase-mediated iodination is greatly enhanced, 100% (Fig. 7). This synergistic effect may be used as a model, mimicking *in vivo* activation of neutrophils. fMLP does not induce iodination on its own at this concentration. In the presence of the different retinoid compounds, the PMA-induced iodination was unaffected, whereas the fMLP-enhanced PMA iodination was significantly inhibited (Fig. 7).

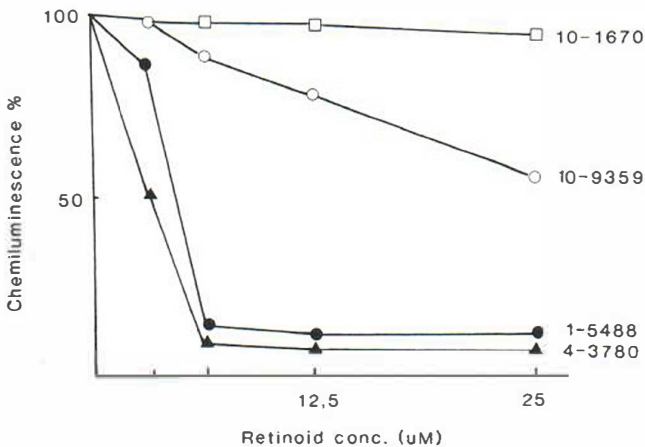


Fig. 4. Effect of different retinoid compounds on chemiluminescence of activated granulocytes using fMLP (10^{-7} M) as stimulus, expressed as % of control cells exposed to 0.1% DMSO.

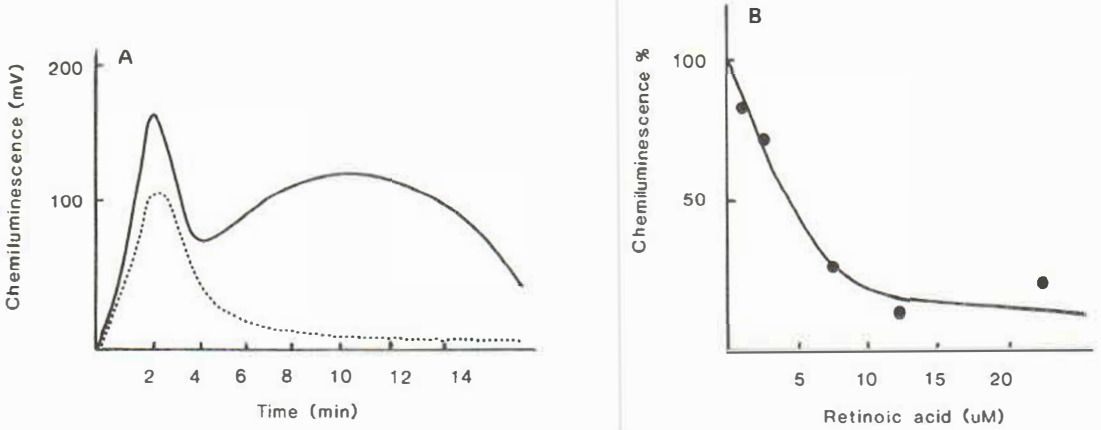


Fig. 5. Effect of retinoic acid on fMLP-induced chemiluminescence (CL) in normal granulocytes. (A) CL pattern of primed cells exposed to fMLP + retinoic acid (---) or to fMLP + 0.1% DMSO (—). (B) Inhibitory effect of different concentrations of retinoic acid.

Particularly retinoic acid, isotretinoin and tretinoin were inhibitory, while Ro 10-1670 was less so and etretinate had virtually no effect.

DISCUSSION

Several reports have suggested that the neutrophil response in psoriasis such as superoxide production (23), chemiluminescence (24), lysosomal enzyme release (25), Fc-receptor activity (26) and cytotoxicity (27) change with the activity of the disease. Earlier investigations of ours, measuring hexose monophosphate shunt activity, have not indicated any difference in oxidative response or myeloperoxidase-mediated iodination between cells from patients with pustular psoriasis and cells from non-psoriatic controls (6). We did not then, however, correlate the cellular response to the degree or course of pustular psoriasis. The present investigation focused on (a) the luminol-enhanced myeloperoxidase-dependent chemiluminescence response in patients with pustular psoriasis and (b) how

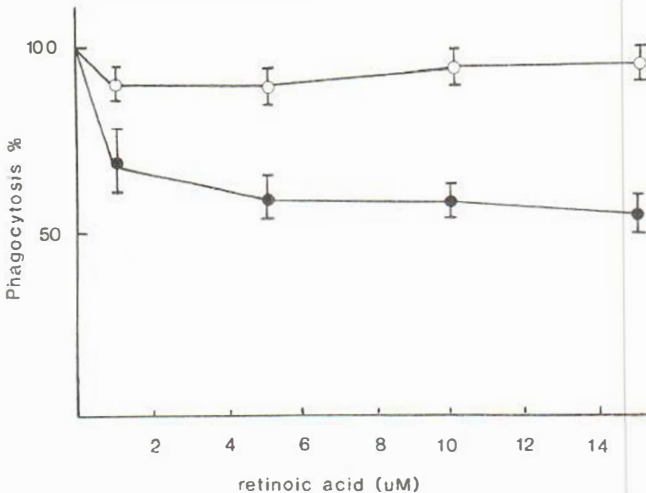


Fig. 6. Effect of different concentrations of retinoic acid on IgG (●) and C3b (○) mediated phagocytosis in normal granulocytes. Vertical bars represent SEM from 3-5 experiments.

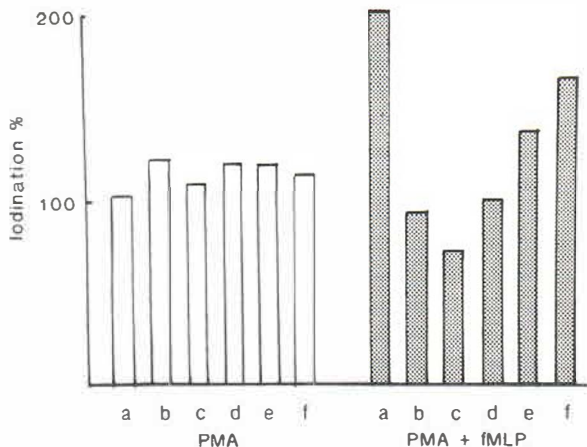


Fig. 7. The effect of different retinoid compounds (25 μ M) on PMA-induced iodination (open bars) and on fMLP-enhanced PMA-induced iodination (hatched bars). No retinoid (a), retinoic acid (b), isotretinoin (c), tretinoin (d), RO-1670 (e) and citreinate (f). The iodination was expressed as % of control cells (= iodination in PMA-stimulated granulocytes exposed to 0.1% DMSO). Mean of two experiments.

different retinoid compounds affect this response. When observing patients with pustular psoriasis it is evident that the chemiluminescence response is greatly enhanced during the initial "acute" phase of pustule formation but close to normal in the non-pustular stages. This enhanced activity is not directly correlated to the leukocytosis in pustular psoriasis, since normal chemiluminescence response was found in other forms of psoriasis with leukocytosis. This contrasts the findings of Schopf et al. (28), who detected enhanced PMA-induced chemiluminescence in psoriasis vulgaris, and those of Ternowitz (29) showing a relationship between clinical status and enhanced chemotaxis in psoriasis. The enhanced chemiluminescence response to fMLP in neutrophils from pustular psoriasis patients showed a two-peak pattern. This pattern is characteristic of cells that have been "activated", i.e. metabolically primed either *in vivo* or *in vitro* (20). Our data thus show that pustular psoriasis cells have been primed *in vivo* and cannot be further primed by preincubation *in vitro*. A similar *in vivo* effect on neutrophils has been observed in patients with bacterial meningitis (Briheim et al. unpubl. obs.).

The mechanism of this process is not clear, but chemotactic factors (20) and endotoxin (30) may affect the cell. Several reports have suggested that serum from psoriatic patients and extract from psoriatic skin contain chemotactic factors (1, 31). However, we could not induce this priming in control cells with pustular psoriasis patient sera and we have in preliminary experiments not been able to detect any increased levels of endotoxin in blood from patients with pustular psoriasis (<20 pg/ml) (Coble et al., unpubl. obs.), as was suggested by others (32).

Table II. IgG and C3bi-mediated phagocytosis^a in neutrophils from pustular psoriasis (P.P.), psoriasis vulgaris (P.V.)

Particles	Neutrophils from		
	PP	PV	Exudative PV
IgG-yeast	108 ± 11	120 ± 16	120 ± 18
C3bi-yeast	115 ± 16	150 ± 15 ^b	122 ± 17

^a Expressed as % of phagocytosis in control cells. Mean ± SEM.

^b $p < 0.01$.

In the present report, neutrophils from patients with pustular psoriasis showed an increased response not only to fMLP, but also to PMA. The increase in PMA response in pustular psoriasis neutrophils is not seen after *in vitro* priming of control cells (20). The enhanced response in pustular psoriasis thus reflects a form of activation, not necessarily coupled only to an increased number of receptors. An enhanced oxidative response may also occur after translocation of the oxidase to the membrane (33). These cells would then in their primed state be more active in releasing toxic oxidative metabolites.

Retinoic acid and isotretinoin caused pronounced inhibition of chemiluminescence and myeloperoxidase-mediated iodination in relevant *in vivo* concentrations (22), whereas etretinate and its metabolite Ro 10-1670 were less inhibitory. Interestingly, the inhibitory effect was evident primarily in primed neutrophils of pustular psoriasis and in primed control cells. This could account for some of the clinical effects of retinoids.

Although etretinate is an effective drug *in vivo* in pustular psoriasis, our results and those of others (10) show that retinoic acid, isotretinoin and tretinoin are the most effective compounds in inhibiting various neutrophil functions. The pronounced effect of these derivatives on chemiluminescence and iodination could be due to the combined effect on superoxide release and degranulation (10).

Retinoic acid furthermore inhibits Fc-mediated phagocytosis, in normal and pustular psoriasis neutrophils, but it is at this stage unclear whether this is an effect on membrane receptors or the subsequent transduction mechanisms. It has also been reported that retinoids may regulate Fc-receptor expression in macrophages (34) and that the enhanced expression of Fc-receptors on the surface of human blood monocytes was abolished by retinoic acid after treatment with interferon (35). Retinoids may influence the biosynthesis of surface glycoproteins, thereby affecting such properties as adhesion, surface recognition and expression of receptors (36, 37). Such modulation of the surface membrane could explain the effect of retinoic acid on phagocytosis. Several retinoid-induced membrane changes have been reported in other cell systems, such as changes in surface membrane glycolipids, glycoproteins (36) and fibronectin (37). Our present observation cannot directly explain the antiinflammatory effects of retinoids in pustular psoriasis, but since retinoids affect not only the oxidative response and phagocytosis, but also the development of receptors for certain chemotactic peptides during differentiation (18), we want to postulate that retinoids have the capacity to interfere with the cellular function of neutrophils *in vivo*.

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