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In Vitro Effects of Ethanol on Polymorphonuclear Leukocyte Membrane Receptor Expression and Mobility

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ABSTRACT. The hampered inflammation and host defense seen in alcoholics may be due to impairment of functional responses of neutrophil polymorphonuclear leukocytes (PMN). We have shown that ethanol inhibits the oxidative metabolism of PMN induced by surface receptor dependent stimuli, such as N-formyl-methionyl-leucyl-phenylalanine (fMLP) and opsonized zymosan. Because the unresponsiveness might be due to reduced numbers of surface receptors, we assessed the expression of CR1, Fc-γ, and fMLP receptors as well as membrane fluidity after treatment of PMN with ethanol *in vitro*. Ethanol impaired the induced expression of CR1 and fMLP receptors to 71% and 51% of control, respectively, but did not affect the resting level of CR1 nor Fc-γ receptor expression. Furthermore, the mobility of cell membrane glycoconjugates was increased by ethanol. However, phagocytosis, a functional response dependent on membrane rheology, was unaffected. Because the results indicated an effect of ethanol on mobilization of receptors from intracellular stores, we assessed lactoferrin release, which was reduced to 59%. Thus, ethanol appeared to hamper the upregulation of PMN surface receptors or functional subsets of those stored in granules. Ethanol also increased the mobility of the cell membrane. These reactions were accompanied by reductions in the functional responses mediated by either class of receptors. BIOCHEM PHARMACOL 51;3:225–231, 1996.

KEY WORDS. polymorphonuclear leukocyte; ethanol; receptor expression; membrane mobility; degranulation; oxidative metabolism

Alcoholics exhibit an increased susceptibility to infections [1, 2], but the mechanisms for this reduced host defence are not known. We and others have demonstrated an inhibitory effect of ethanol on the oxidative metabolism and adhesive responses of PMN‖ *in vitro* [3–6]. Because these functional responses were mediated by stimuli that bind to membrane surface receptors, it might be assumed that ethanol interferes with the expression or function of such receptors. [4, 5, 7].

Phagocytosis is mediated by the Fc-γ receptor and the complement receptor, which recognize C3 fragments (CR1, CR3) [8]. Furthermore, oxidative, chemotactic, and adhesive responses are induced *via* activation of, for instance, the fMLP receptors as well as the complement receptor CR3. These receptors are expressed on the surface of quiescent neutrophils, but are also stored in secondary granules (fMLP and CR3) or other intracellular vesicles (CR1). They can be mobilized to the surface upon stimulation of the cell, whereby the content of specific granule (e.g. lactoferrin) is extruded [9, 10].

The possibility that ethanol may interfere with surface receptor expression has been explored by Yuli *et al.* [7], who reported that *in vitro* treatment with 2.5% ethanol did not affect the binding of ^{3}H-fMLP to the neutrophil. Likewise, MacGregor *et al.* [5] observed that ethanol did not inhibit the fMLP-induced specific granule release from PMN when preincubated with 2.5% ethanol for 20 min. However, ethanol has been shown to reduce the number of functional Fc-γ receptors [11] in human monocytes, and to inhibit the fMLP-induced elastase release from azurophilic granules of PMN [3]. We have recently shown that the number of CR3 is reduced after treatment *in vitro* with ethanol [4]. Furthermore, the affinity and fundamental activity of the receptor for formylated oligopeptides have been shown to be altered by aliphatic alcohols. These alcohols (e.g. butanol and pentanol) were shown to enhance the binding of the formylated oligopeptides to human PMN, either by increasing the number of receptors [12, 13] or by changing their affinity [7]. The shift in binding affinity was proposed to correlate with the degree of membrane fluidization induced by these alcohols [7].

Changes in membrane rheology have been shown to modify

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‖ *Abbreviations:* PMN, neutrophil polymorphonuclear leukocyte; fMLP, N-formyl-methionyl-leucyl-phenylalanine; CR1/CR3, complement receptors; HBSS, Hanks' balanced salt solution; FITC, fluroscein-isothiocyanate-conjugated; LCL, luminol-enhanced chemiluminiscence; ELISA, enzyme-linked immunoassay; BSA, bovine serum albumin; DMSI, dimethyl-suberimidate; S-WGA, succimylated wheat germ agglutinin; FRAP, fluorescence after photobleaching; D, diffusion constant; R, percent mobile receptors; Kd, dissociation constant; MFI, mean fluorescence intensity.

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binding characteristics of receptors [14, 15] and receptor-induced metabolic processes [7, 16]. Concerning the effect of ethanol on membrane fluidity, it is a commonly held view that ethanol *in vitro* renders membranes more fluid [7, 17].

This report concerns the effect of ethanol on PMN membrane glycoconjugate mobility, specific granule release, and the expression of membrane receptors for fMLP, the Fc-part of IgG and C3. The question we asked was whether the inhibitory effect of ethanol on PMN functional responses was due to a biophysical effect on the cell plasma membrane resulting in altered receptor expression.

MATERIAL AND METHODS

Chemicals

The following chemical reagents were used: HBSS and Hepes buffered RPMI 1640 (Gibco Ltd, Paisley, UK); purified human IgG (Kabi, Stockholm, Sweden), Percoll (Pharmacia, Uppsala, Sweden); NH_4Cl 'Lysing reagent' (Ortho Diagnostic Syst., Westwood, NJ); FITC rabbit antimouse IgG-F(ab')$_2$ and mouse monoclonal anti-CR1 antibody (MoAb-CR1: Dakopatts AS, Glostrup, Denmark); 96-well microtiter plates (NUNC Immunoplate 1 96F, Roskilde, Denmark); IgG fraction of antihuman lactoferrin and peroxidase-conjugated IgG antilactoferrin antibodies (Cappel Laboratories, West Chester, PA); purified human lactoferrin (Calbiochem, La Jolla, CA); ^{3}H-fMLP (55.2 Ci/mmol: New England Nuclear, Boston, MA); Versilube F 50 silicon fluid (General Electric Co, Waterford, NY); and NCS tissue solubilizer (Amersham Corp, UK). All other chemicals were from Sigma Chemical Co. (St Louis, MO).

The concentrations of ethanol used here are given as vol/vol percentages. Thus, 1% ethanol corresponds to 7.9 mg ethanol/mL or 0.17 M.

Cell Isolation

The choice of leukocyte isolation method is difficult. Any method involving many steps might prime the PMN. For each experiment, we chose the gentlest method given the demands (e.g. PMN purity). Three different cell preparation methods were used (A, B, and C):

A. Percoll Separation. Heparinized blood samples were obtained from healthy staff members, none of whom was on medication. PMN were isolated by a one-step Percoll technique [18], followed by lysis of residual erythrocytes with 0.155 M ammonium chloride. With this technique, there was approximately one platelet per ten granulocytes [19]. Cells were suspended in HBSS (at pH 7.45) and kept at 4°C until use, followed by 15-min incubation at 37°C. This method was used for assessments of luminol-enhanced chemiluminiscence (LCL) and other assays when the contamination of erythrocytes and platelets must be kept at a minimum.

B. The NH_4Cl Lysing Procedure. EDTA blood was collected from healthy blood donors not taking any drugs. The erythrocytes were lysed in 100 μL portions by dilution in 2 mL of 0.15 M NH_4Cl 'lysing reagent.' The leukocytes were then isolated at 4°C by centrifugation at 300 × g for 5 min. This method was employed in flow cytometry to minimize upregulation of surface receptors during cell preparation.

C. Ficoll Paque Separation. Isolation was done essentially according to Boyum [20].

Lactoferrin Release

PMN, isolated according to method A, were treated with ethanol or HBSS for 10 min followed by an incubation with HBSS or 100 nM fMLP at 37°C for 10 min. The suspensions were centrifuged and the supernatants analyzed as described below. The release of lactoferrin was assessed by an enzyme-linked immunoassay (ELISA) [21]. Briefly, 96-well microtiter plates (NUNC Immunoplate) were coated with rabbit IgG antihuman lactoferrin in coating buffer. The plates were washed 4 times with PBS/Tween 20 and tapped dry. Lactoferrin standards and samples were added in 1% BSA/PBS. The plates were incubated for 90 min at 37°C, washed 6 times with PBS/Tween 20 and tapped dry. The peroxidase-conjugated IgG antilactoferrin was added. After 15 min, the reaction was stopped with H_2SO_4. The plates were read on a microtiter reader at O.D. 490 nm. Calculations were performed as described [21].

FMLP-Receptor Expression

PMN, isolated according to method A, were initially incubated in HBSS with 1% ethanol at 37°C for 10 min; subsequently ^{3}H-fMLP binding was performed as previously described [22]. Briefly, ^{3}H-fMLP alone (= total binding), or ^{3}H-fMLP plus a 1000-fold excess of nonradioactive fMLP (= nonspecific binding) were added to suspensions of PMN; the final cell concentration was 5×10^6/mL in HBSS without Ca^{2+} and Mg^{2+}. In addition, at the end of the 30-min incubation, an aliquot of cells from the total binding tube was added to a vial with a 100-fold excess of nonradioactive fMLP to allow surface-bound ^{3}H-fMLP to be released from the cells (= displaceable binding). Bound and free peptides were separated by centrifugation of cells through silicon oil. The oil was then removed and the pellets added to scintillation vials containing 1 mL NCS and shaken overnight on a platform rocker. Subsequently, 0.034 mL glacial acetic acid and 10 mL scintillation fluid were added and the samples counted in a Rack Beta scintillation counter (LKB-Wallac OY, Turku, Finland). Calculations were performed as previously described [21]. Scatchard plots of the experimental data were fitted by regression analysis and the dissociation constants and receptor number estimated from fitted slopes and x-intercepts. Corresponding estimates were also made by a computer-modeling method using a nonlinear least squares algorithm [23].

Four separate experiments with different donors were performed; however, at each occasion PMN from one individual was both ethanol-treated and control.

C3b-Receptor (CR1) Expression

Leukocytes were prepared with method B. The leukocytes were washed once in cold 0.15 M phosphate-buffered saline, pH 7.4, supplemented with 0.1 mM EDTA and 0.02% sodium-azide (PBS-EDTA). Two different methods were then used.

METHOD 1. The PMN were preincubated for 5 min in Tris-HBSS-buffer at 4°C or 37°C for 5 min and then, with or without ethanol (1%), at either temperature for 10 min. The cells were then incubated with Hepes-RPMI with various concentrations of fMLP for another 15 min, washed in cold PBS-EDTA, and resuspended in 100 µL PBS-EDTA. Ten µL unconjugated MoAb-CR1 was added and the cells were incubated cold for 30 min, washed twice with PBS-EDTA, and incubated cold and dark for another 30 min with 100 µL FITC-rabbit antimouse IgG-F(ab')$_2$, diluted 1:20 in PBS-EDTA. After washing, the cells were resuspended in PBS-EDTA and, finally, examined in an Ortho spectrum III flow cytofluorometer (Ortho Diagnostics Syst. Inc., Westwood, NJ). The granulocyte field was examined as previously described [24].

METHOD 2. The cells were resuspended in Hepes-RPMI and Hepes-RPMI with various concentrations of ethanol (0.05%–1.6%). They were then incubated at 4°C and 37°C, respectively for 10 min. The cells were then incubated another 15 min with different concentrations of fMLP, washed once, and from then on treated as in Method 1 from the addition of the MoAb-CR1.

Fc-γ-Receptor Expression

Purified human IgG were aggregated by incubation in a 30-fold molar excess of DMSI in 0.2 M tris-HCl buffer, pH 8.5, for 2 hr at 30°C with occasional stirring. The aggregates were dialyzed against 0.05 M carbonate buffer, pH 9.5, and conjugated with FITC by incubating the protein (5 mg/mL) overnight at 4°C in a 0.05 M carbonate buffer, pH 9.5, containing 0.1 mg FITC per mL (BBL, Cockeysville, U.S.A.). Free FITC molecules were eliminated by fractionation on a PD-10 column (Pharmacia, Uppsala, Sweden). The final F/P ratio was 5.3 for DMSI-aggregated IgG [25]. PMN isolated according to method B were washed once in ice-cold 0.15 M phosphate-buffered saline supplemented with 0.1 mM EDTA (PBS-EDTA) and then resuspended in 0.05 mL of FITC-aggIgG (100 µg/mL). These suspensions were incubated for 60 min in ice bath and then washed 3 times in PBS-EDTA. The leukocytes were finally resuspended in 1 mL cold PBS-EDTA and analyzed in an Ortho Spectrum III flow cytofluorometer (Ortho Diagnostic Syst. Inc., Westwood, NJ).

Chemiluminescence

The oxidative metabolism of PMNs was assessed as LCL. Briefly, PMN purified according to method A were mixed with luminol (0.17 mM) to a final concentration of 1.5×10^6 PMN/mL. The pH was kept at 7.6–7.8. After assessing spontaneous LCL, reagents were added and LCL was followed continuously with a Luminometer 1050 (LKB, Bromma, Sweden) [3]. The results are given as net LCL (i.e. stimulated minus spontaneous light emission) in mV for (at least) triplicates. Ethanol-treated PMNs showed the same background LCL as untreated cells.

Phagocytosis

Yeast C3b particles were prepared as previously described [26]. Leukocytes were provided by using method B. Leukocyte pellets were washed once in Gey's medium and incubated for 15 min at 37°C with 200 µL FITC-labeled C3b-coated yeast particles, suspended in Gey's buffer at a concentration of 5×10^6 particles/mL. The suspensions were diluted in cold PBS supplemented with EDTA (PBS-EDTA) and examined in the Ortho spectrum III flow cytometer [26]. Using this phagocytic assay, it is possible to differentiate between attached and ingested particles by adding dyes (e.g. trypan blue) that quench the fluorescence of attached but not ingested particles due to dye exclusion by viable cells [26].

Receptor Mobility

Lateral diffusion of fluoresceinated succinylated wheat germ agglutinin (S-WGA-FITC)-labeled membrane glycoconjugates was measured using FRAP [27–29]. The PMN were separated according to method C. They were attached to glass and labeled with fluoresceinated succinylated wheat germ agglutinin (10 µg/mL) for 2 min at room temperature. The principles for FRAP have been described in detail elsewhere. Briefly, data acquisition and handling was done with microcomputers, allowing two modes of measurements, RAPID-FRAP [27–29] and SLOW-FRAP [28–29]. In FRAP, fluorescence is sampled at short time intervals (0.05 sec) to assess whether a rapidly diffusing component such as a lipid is present. SLOW-FRAP is useful for studies of slow-diffusing species (e.g. proteins). The S-WGA labelled cells were illuminated and bleached through a circular slit placed in a Zeiss Universal microscope (Zeiss, Oberkochen, Germany) via a x63 oil immersion objective, yielding a bleached area radius (ω) of approximately 0.9 µm in the plane of the membrane. The laser used was a Spectra-Physics Argon Laser (Type 2020-03, Mountain View, CA), run in Current mode (20A). A bleached pulse of 250 ms was used. For RAPID-FRAP, the recovery was studied for 5 sec, and for SLOW-FRAP, for 90 sec. The diffusion constant (D) and the percent recovery after photobleaching (R = percent mobile receptors) were calculated according to established principles [27].

Statistical Analyses

Student's *t*-test for paired and unpaired samples.

RESULTS

Lactoferrin Release

The spontaneous release of lactoferrin was not significantly affected by treating neutrophils with ethanol (1%; $P > 0.2$).

However, the release of lactoferrin induced by fMLP (100 nM) was inhibited by 1% ethanol, being only 59 ± 9% ($n = 4$) of the release by control cells, viz. 40 ± 4 and 24 ± 3 μg/10^7 PMN, respectively ($P < 0.01$).

Because the release of a specific granule product is accompanied by translocation of formylpeptide- and C3-receptors from specific granules to the plasma membrane [30], we studied the effect of ethanol on the expression of these receptors.

FMLP-Receptor Expression

Figure 1 depicts the results of ^{3}H-fMLP binding studies with and without the presence of ethanol. In each experiment, the total specific binding of ^{3}H-fMLP was impaired in the presence of ethanol. Using Scatchard analysis, it was evident that the binding plots obtained were curve-linear, both for ethanol and control samples, but could also be fitted to two linear functions. Therefore, regression analysis was done, assuming a two-receptor model with different affinities. When the dissociation constant (K_d) was calculated for the two different parts of the curve-linear graph, neither the low-affinity part nor the high-affinity part had a K_d that differed significantly between the control and ethanol-treated group (Table 1). There was, however, a significantly ($P < 0.05$) impaired number of low-affinity binding sites per cell in the presence of ethanol (Table 1). The number of high-affinity sites was unaffected by the presence of ethanol ($P > 0.2$) (Table 1).

CR1 Expression

The spontaneous expression of CR1 at 4°C, measured as MFI, was unaffected by the presence of ethanol (methods 1 and 2). Thus, MFI at 4°C was 51 ± 8 in control PMN and 57 ± 11 in presence of 1% ethanol. The net increase of MFI in cells incubated at 37°C was dose-dependently inhibited with ethanol (Fig. 2). Ethanol also gradually inhibited the fMLP (5 and 50 nM)-induced increase in MFI (Fig. 2).

Fc-γ Receptor Expression

There was no difference in spontaneous Fc-γ receptor expression on PMN at 4°C in the presence of ethanol (1.6%) compared to control cells ($P > 0.5$). Moreover, incubation at 37°C did not significantly ($P > 0.5$) increase Fc-γ receptor expression, either in control cells or in ethanol-treated cells. FMLP (5 nM and 50 nM) did not significantly increase Fc-γ receptor expression ($P > 0.2$). Ethanol had no influence on fMLP-induced receptor expression (Table 2).

The use of 1.6% ethanol instead of 1% was for technical reasons. However, there was no change in Fc-γ receptor expression in the presence of either 1.6% or 0.8% ethanol. These results indicate that 1% ethanol has no influence on Fc-γ receptor expression.

Luminol-Enhanced Chemiluminescence

For these studies, we chose a stimulus that activates the PMN *via* the C3b receptor (i.e. opsonized zymosan). The effect of 1% ethanol on oxidative metabolism, measured as LCL induction, by this stimulus was significantly lower (77 ± 2 mV) than in control cells (115 ± 4 mV) ($n = 3$, $P < 0.01$).

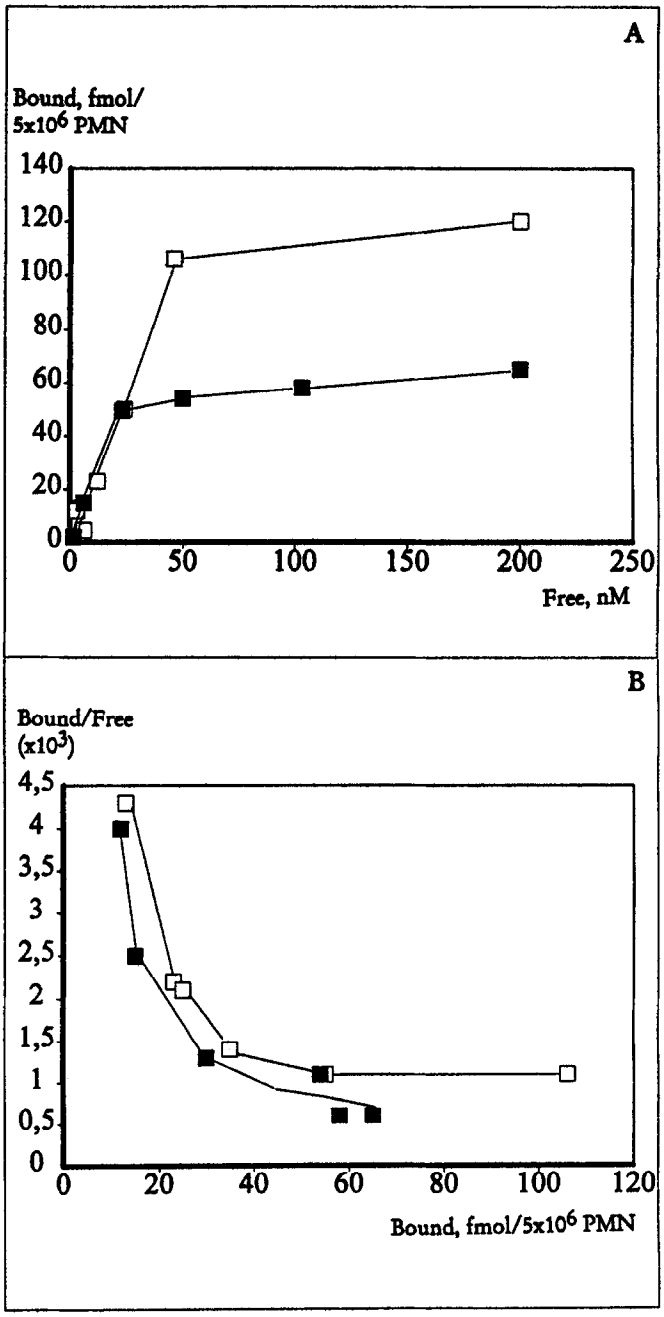

FIG. 1. Binding of ^{3}H-fMLP to PMN previously incubated in the absence (□) or in the presence (■) of 1% ethanol. Data presented are that of a representative experiment. (A) shows the total specific ^{3}H-fMLP binding to PMN. (B) shows a Scatchard plot of ^{3}H-fMLP binding to PMN. Scatchard plots of the experimental data from ^{3}H-fMLP binding studies were fitted by regression analysis and a computer-modeling method using a nonlinear least-squares algorithm.

Phagocytosis

The ability of PMN to attach to and ingest yeast-C3b was not affected by the presence of 1% ethanol. The percentage of

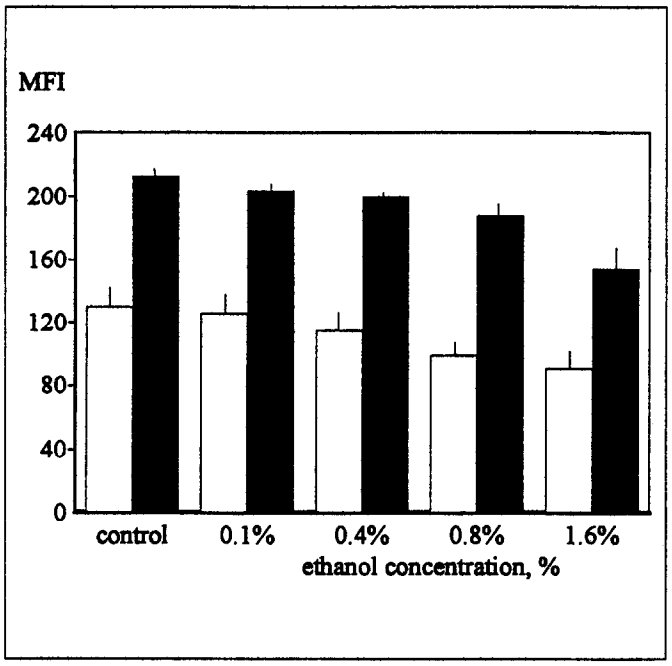

FIG. 2. The number of CR1 measured as mean fluorescence intensity (MFI) in absence or in presence of various concentrations of ethanol. The bindings were carried out without stimulus (white bars) or in the presence of 50 nM fMLP (black bars) at 37°C. Paired Student's *t*-test of the data obtained from experiments revealed a significant inhibition of the MFI in the presence of 0.8% and 1.6% ethanol ($P < 0.001$) and no difference in the presence of lower concentrations of ethanol ($P > 0.05$). Mean ± SE values.

PMN interacting with attached and/or ingested yeast C3b was 32 ± 2% in control cells and 30 ± 1% in presence of 1% ethanol. In control and ethanol-treated PMN 65% and 61%, respectively of the PMN interacted with yeast-C3b ingested particles.

Membrane Fluidity

It has been proposed that the transduction mechanism for the various biological activities of the chemotactic receptors can be modulated by membrane fluidizers. We, therefore, studied the effect of ethanol on the lateral diffusion of S-WGA-FITC-labeled membrane glycoconjugates. Ethanol (1%) treatment increased the glycoconjugate diffusion coefficient (D) approximately 2-fold ($P < 0.01$), whereas the mobile fraction (R) remained constant (Table 3).

DISCUSSION

This study explores the possibility that the inhibitory effects of ethanol on PMN functional responses, shown in this as well as in previous reports [3, 4, 31], may be due to a fluidizing effect on the cell plasma membrane followed by reduced receptor expression. We show here that ethanol increased the lateral mobility of membrane glycoconjugates, as reflected by wheat germ agglutin experiments. However, ethanol had no general effect on receptor expression, although the upregulation of the fMLP and CR1 receptors was impaired.

After the initial interaction between a ligand (e.g. fMLP) and its specific membrane receptor, the ligand-receptor complexes are believed to be internalized *via* endocytos [32, 33]. Simultaneous upregulation of unoccupied receptors is thought to occur from intracellular reservoirs [for a review, see 9]. Like the fMLP receptor, CR3 have recently been shown to be stored in specific granules [10, 34, 35], and CR1 are stored in other intracellular vesicles [10]. By contrast, the Fc-γ receptor has no intracellular store [36].

A model has been proposed for the signal transduction mechanisms and cellular responses conveyed by chemotactic factor receptor activation at low and high concentrations of ligands. Low chemoattractant doses mediate chemotactic functions (e.g. morphological polarization, cytoskeletal rearrangements, stimulated adhesion, and locomotion). Approximately 10-fold higher concentrations are needed for microbicidal and/or secretory functions (e.g. lysosomal enzyme secretion and induction of a respiratory burst) [7, 12, 37]. Despite the impaired upregulation of fMLP receptors in the presence of ethanol, as shown in this report, we have previously presented evidence that fMLP-induced chemotaxis, adherence to plastic, and membrane potential are unaffected by ethanol [4]. On the contrary, the fMLP-induced oxidative metabolism [3] and, as demonstrated in this study, degranulation are impaired. These results are in agreement with the model with two different affinity states of the fMLP receptors presented above. Thus, the inhibition of fMLP-induced functional responses by ethanol can be explained by an impaired upregulation or an increased internalization of low-affinity state fMLP receptors. By contrast, the high-affinity state receptors and responses mediated by them were unaffected by ethanol. Ethanol had no effect on the binding affinities within the two groups. In previous reports [7, 38] 2.5% ethanol has been shown to have no significant effect on the binding of fMLP to PMN. Other studies [12, 13, 38] have observed that certain aliphatic alcohols, particularly n-propanol and n-butanol, can increase the number of N-formylated peptide receptors on PMN. The stud-

TABLE 1. The effect of 1% ethanol on the dissociation constants and the number of fMLP receptors on human PMN

	Dissociation constant in nM		Number of receptors/cell	
Receptor affinity state	low	high	low	high
Control PMN	109 ± 7.6	3.8 ± 0.6	22,680 ± 1,324	3,048 ± 560
Ethanol-treated PMN	113 ± 5.2	3.4 ± 0.5	9,628 ± 870	3,600 ± 480

Mean ± SE values for 4 separate experiments in triplicate.

TABLE 2. The expression of Fc-γ receptors in PMN in absence or in presence of 1.6% ethanol

Treatment	Control	1.6% ethanol
4°C, no stimulus	29 ± 0.4	30 ± 1
37°C, no stimulus	28 ± 3	28 ± 2
37°C, 5 nM fMLP	32 ± 4	29 ± 2
37°C, 50 nM fMLP	36 ± 6	30 ± 4

Mean ± SE values for 4 separate experiments.

ies of aliphatic alcohols on the affinity of N-formylated peptide receptors have given disparate results; some presented no effect on the affinity state [12, 13] and others showed an increased affinity [38].

The upregulation of CR1 and, as previously shown for CD18 (part of CR3) [4], was impaired in the presence of ethanol. CR1 exists only in one affinity state [39, 40]. A functional response mediated *via* the CR1, the oxidative metabolism, was also hampered in presence of ethanol (this report). The mechanism could be an impaired upregulation of the CR1 in the presence of ethanol. The reduced expression of membrane receptors did not indicate a nonspecific action of ethanol on surface molecules, because neither the resting level of CR1 nor the expression of the Fc-γ-receptor was impaired in the presence of ethanol. These results point toward an effect of ethanol on degranulation, which results in a reduced upregulation of receptors, rather than in increased internalization of receptors.

Ethanol (1%) increased the lateral mobility of WGA-labeled glycoconjugates in the PMN membrane, as reflected by the fluorescence recovery after photobleaching experiments. These results are in agreement with previous reports on various other cell types [7, 17]. The fluidizing effect of ethanol on the plasma membrane might change the expression of the different membrane receptors and then modify the functional responses of the human PMN. Further, despite the fluidizing effect of ethanol on the cell membrane, processes that are dependent upon cell membrane rheology [41, 42], such as phagocytosis, were unaffected by ethanol.

Incidentally, ethanol impaired degranulation, oxidative metabolism, and receptor upregulation (i.e. responses dependent on calcium [31, 43–45]), whereas Ca^{2+}-independent processes such as CR3-phagocytosis [46] and basal membrane receptor expression were unaffected. This could suggest an effect of ethanol on the generation of second messengers. A possible explanation is the previously reported impaired influx of calcium across the cell membrane and the inhibited generation of diacylglycerol and phosphatidic acid in human PMN in the presence of ethanol [31].

To conclude, ethanol hampered the upregulation of PMN surface receptors or functional subsets of those stored in granules and increased the mobility of the cell membrane. The reactions were accompanied by a reduction in the functional responses that these receptors mediate. These factors might, at least in part, explain the increased morbidity and mortality in infections after ethanol administration or abuse.

TABLE 3. Effects of ethanol (1 and 5%) on the lateral diffusion of wheat-germ agglutinin-labelled glycoconjugates in human PMN

Ethanol concentration	Diffusion constant D (10^{-10} cm^2s^{-1})	Mobile fraction, R (%)	*n*
0%	1.1 ± 0.2*	32 ± 3	26
1%	1.9 ± 0.5*	29 ± 4	11
5%	0.8 ± 0.2	37 ± 7	6

* $P < 0.01$. Mean and SE values.

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