

INVOLVEMENT OF MEMBRANE CALCIUM IN THE RESPONSE OF RABBIT NEUTROPHILS TO CHEMOTACTIC FACTORS AS EVIDENCED BY THE FLUORESCENCE OF CHLOROTETRACYCLINE

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ABSTRACT

We have utilized the fluorescent chelate probe chlorotetracycline to investigate the possible involvement of membrane calcium in the response of rabbit peritoneal neutrophils to chemotactic factors. Two chemotactic factors, the small molecular weight fragment of the fifth component of complement C5a and the synthetic peptide formyl-methionyl-leucyl-phenylalanine (F-Met-Leu-Phe), were tested and found to decrease the fluorescence of cell-associated chlorotetracycline in a manner strongly suggesting stimulus-induced displacement of membrane calcium. The time-course, concentration dependence, and receptor specificity of the calcium redistribution induced by the stimuli are consistent with its early role in the initiation of the various neutrophil functions. F-Met-Leu-Phe and C5a appear to interact with the same pool of membrane calcium and to release it to the cytoplasmic side of the plasma membrane. Intracellular calcium then binds back to the membrane(s) from where it can be displaced by additional stimulation. The release of membrane calcium, experimentally defined here, appears to play a central role in the initiation of the various neutrophil functions.

KEY WORDS neutrophils · F-Met-Leu-Phe · C5a · membranes · calcium · chlorotetracycline

Evidence from various types of investigations indicates that in the neutrophil the effects of chemotactic factors and secretagogues are mediated by variations in the levels of free intracellular calcium (2, 4, 13, 17–19, 23, 24, 27, 29). In this, the neutrophil is like many other nonmuscle cells (10, 11, 25).

The calcium required for the activation of the neutrophils may come from the extracellular medium and/or the intracellular stores of calcium.

Chemotactic factors and the secretagogues induce changes in the plasma membrane permeability of the neutrophils to calcium (4, 13, 17, 18, 20). In addition, these stimuli require extracellular calcium to produce optimum chemotactic (3, 13, 33) and secretory (23, 30) responses. These two sets of results make it likely that to a certain extent neutrophils depend on the extracellular pool of calcium.

However, when necessary, neutrophils can mobilize calcium from some, as yet undefined, intracellular stores. The removal of extracellular calcium only shifts the dose-response curve for lyso-

somal enzyme release from rabbit neutrophils but does not abolish their secretory responsiveness (20, 30). Under some circumstances cell locomotion and/or orientation does not depend on extracellular calcium (16, 34). The relative contribution of these two sources of calcium depends on the concentration of extracellular calcium and on the level of stimulation (23, 24).

We wish now to report the results of a study of the intracellular pool(s) of calcium of the neutrophils utilizing the fluorescence of cell-associated chlorotetracycline. Chlorotetracycline is a hydrophobic molecule which partitions into cellular membranes and whose fluorescence characteristics are dependent on the level of divalent cations chelated to it, hence the designation as a fluorescent chelate probe (5, 6, 15). It is thus an extremely useful probe with which to investigate possible intracellular events related to divalent cations and their interactions with membranes such as mitochondrial and endoplasmic reticulum membranes (5-9, 15, 31). The experiments to be described clearly establish the existence of a pool of membrane calcium which can be mobilized both by the synthetic chemotactic peptides and by C5a and which thus appears to be central to the physiological regulatory mechanisms of the neutrophils.

MATERIALS AND METHODS

Cell Preparation and Handling

Rabbit peritoneal neutrophils were collected by drainage 12-16 h after the peritoneal injection of 400 ml of sterile saline containing 0.1% glycogen. They were then washed and resuspended in Hanks' balanced salt solution containing 10 mM *N*-2-hydroxyethylpiperazine-*N'*2'-ethane sulfonic acid (HEPES), pH 7.3 (30). Magnesium and bovine serum albumin were omitted to minimize chemotactic factor-induced cell aggregation (21) and extracellular fluorescence (8), respectively. All the experiments were carried out at 37°C and at a cell concentration of 3.0×10^6 cells/ml. A 10-min preincubation at 37°C preceded all further experimental manipulations.

Fluorescence Measurements

All fluorescence measurements were made in a Perkin-Elmer MPF-2A fluorescence spectrophotometer (Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.) equipped with a temperature-controlled cuvette holder and a laboratory-built electric stirrer. The measurements were made under two sets of conditions which will be referred to as uptake and efflux experiments, the protocols of which follow.

UPTAKE MEASUREMENTS: In these experiments,

chlorotetracycline (10-20 μ M) was added first to thermally equilibrated cell suspensions (3.0×10^6 cells/ml). When desired, calcium (0.5 mM) was added a few minutes after the fluorescent probe. The cell suspensions were then transferred to the spectrophotometer and their fluorescence was monitored on a recorder. The various additions (chemotactic factors, inhibitors, etc. . .) were then made at the desired times without removing the cells from the solutions containing the fluorescent probe. The effects of the various additions on the fluorescence were monitored continuously on the recorder.

EFFLUX MEASUREMENTS: In this experimental protocol the cells (3.0×10^6 cells/ml) were incubated for 30 min with chlorotetracycline (10-100 μ M) in the presence or absence of 0.5 mM calcium. This time is sufficient for the fluorescence to reach a steady-state level (see Fig. 1). The cells were then washed twice and resuspended at 3.0×10^6 cells/ml in thermally equilibrated Hanks' balanced salt solution containing no chlorotetracycline in the presence or absence of calcium (0.5 mM). The fluorescence intensity was found to decrease in a biphasic manner, an initial rapid fall in fluorescence followed by a slower sustained loss as previously described (8, 19). The desired additions were all made during the second phase of the fluorescence loss, and their effects on the fluorescence intensity were monitored on a recorder.

Unless specified otherwise, the excitation and emission wavelengths were set at 390 and 520 nm, respectively.

The excitation and emission slits were adjusted as required between 6 and 10 nm.

Control experiments showed that chlorotetracycline at concentrations up to 100 μ M did not affect the functional responsiveness of the neutrophils as judged by their ability to release lysosomal enzymes when exposed to chemotactic factors and cytochalasin B (28-30).

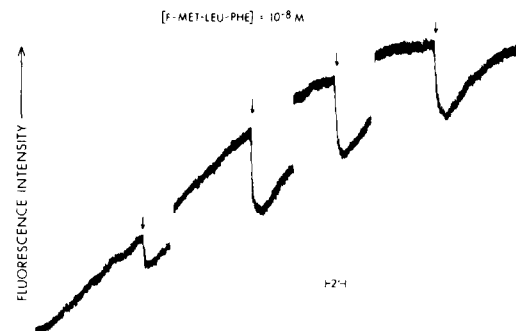


FIGURE 1 Time-course of the uptake of chlorotetracycline into rabbit neutrophils and the effect of F-Met-Leu-Phe. Chlorotetracycline (10 μ M) was added first to thermally equilibrated cell suspensions (3.0×10^6 cells/ml) followed 1 min later by calcium (0.5 mM). The cells were then divided among the four spectrophotometer cuvettes, transferred to the cuvette holder, and the fluorescence intensity was monitored. F-Met-Leu-Phe (10^{-8} M) was successively added to each of the four cuvettes at the times indicated by the arrows.

No dependence on the concentration of chlorotetracycline could be detected.

Chemicals

Chlorotetracycline, oxytetracycline, and perylene were obtained from Sigma Chemical Co. (St. Louis, Mo.). 1-Anilino-8-naphthalene sulphonate was purchased from the Eastman Organic Chemicals Div., Eastman Kodak Co. (Rochester, N. Y.). Chlorotetracycline was added to the cells either directly in powder form (100 μ M) or as a dilution from a 10^{-2} stock in dimethylsulfoxide made on the day of the experiment. Cytochalasin B was obtained from Aldrich Chemical Corp., Inc. (Milwaukee, Wis.).

The synthetic peptides, formyl-methionyl-leucyl-phenylalanine (F-Met-Leu-Phe) and 5-butoxycarbonyl-phenylalanyl-leucyl-phenylalanyl-leucyl-phenylalanine (boc-Phe-Leu-Phe-Leu-Phe) were generously provided by Dr. R. J. Freer (Medical College of Virginia, Richmond, Va.). The chemotactically active fragment of the fifth component of complement (C5a) was generated in whole serum and purified according to the method of Fernandez and Hugli (12).

EGTA and EDTA were purchased from Sigma Chemical Co.

RESULTS

Effect of Chemotactic Factors on the Cell-Associated Fluorescence of Chlorotetracycline

The effects of the chemotactic factor F-Met-Leu-Phe on the fluorescence of cell-associated chlorotetracycline were first examined. Fig. 1 illustrates the results of uptake experiments performed in the presence of a 0.5 mM Ca^{2+} in which F-Met-Leu-Phe (10^{-8} M) was added at different times after chlorotetracycline. A time-dependent increase in the fluorescence of chlorotetracycline is observed and reaches an apparent plateau or steady-state in ~ 25 min. The addition of F-Met-Leu-Phe causes a rapid decrease in the fluorescence of cell-associated chlorotetracycline. This decrease in fluorescence reaches its maximum within a minute after the addition of the chemotactic factor and is followed by a slower recovery of the fluorescence. As can be seen in Fig. 1, the fluorescence intensity comes back up to the steady-state level of control, untreated cells within 6–10 min. It should be noted that <10 min is required for chlorotetracycline to reach the membrane compartment which is sensitive to F-Met-Leu-Phe. In addition, the magnitude of the fluorescence changes appears to be constant after 20-min incubation with chlorotetracycline. Essentially similar results are obtained with C5a.

We have examined next the role of extracellular divalent cations in the fluorescence changes caused by chemotactic factors in uptake experiments. As previously reported (18), extracellular calcium is not required for the chemotactic factor-induced increase in the rate of loss of the fluorescence of cell-associated chlorotetracycline as measured in cells preloaded with chlorotetracycline. It was of particular interest to determine whether or not the recovery phase of the fluorescence changes induced by chemotactic factors in the uptake experiments was dependent on the presence of extracellular divalent cations. Fig. 2 shows the results of an experiment in which chlorotetracycline was added to cells incubated in the absence of added extracellular divalent cations and in which fluorescence was monitored. It can first be seen that the uptake curve of chlorotetracycline illustrated in Fig. 2 is very similar to that shown in Fig. 1, i.e., in the presence of 0.5 mM Ca^{2+} . Steady-state appears, however, to be reached somewhat faster in the absence of extracellular calcium (15 vs. 25 min). Although not evident from the figures, the magnitude of the fluorescence recorded in the absence of extracellular calcium was found to be smaller than in the presence of calcium. The addition of F-Met-Leu-Phe (10^{-8} M) to the neutrophils causes, as described in Fig. 1, a rapid decrease in the steady-state fluorescence followed by a slower recovery phase. This pattern of changes in

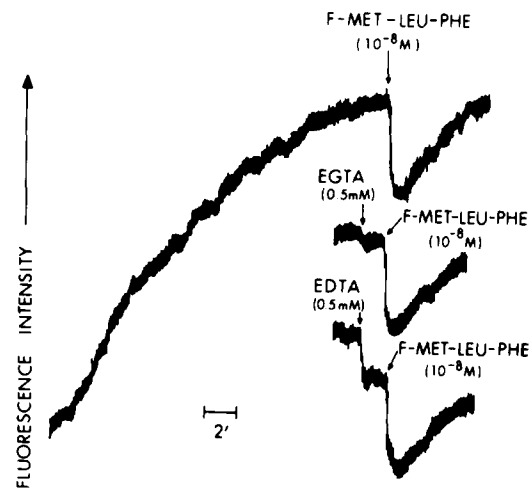


FIGURE 2 Effect of F-Met-Leu-Phe on the steady-state fluorescence of cell-associated chlorotetracycline in the absence of extracellular divalent cations. The cells were treated as in Fig. 1 except that no calcium was added. EDTA, EGTA, and F-Met-Leu-Phe were added at the steady-state level of the fluorescence increase curve.

the fluorescence can, in addition, be seen to be essentially unaffected by the presence of 0.5 mM EDTA and/or EGTA. The small decreases in fluorescence induced by EDTA and to a lesser degree by EGTA are most probably due to the chelation of the contaminating levels of Mg^{2+} and Ca^{2+} in the extracellular medium. Fig. 2 thus conclusively demonstrates that the fluorescence changes induced by chemotactic factors reflect intracellular events which are independent of the ionic nature of the extracellular medium.

The fluorescence of cell-associated oxytetracycline, a divalent cation-insensitive fluorescent probe structurally very similar to chlorotetracycline (8), 1-anilino-8-naphthalene sulfonate, and perylene were found not to be affected by F-Met-Leu-Phe (data not shown).

Effect of Boc-Phe-Leu-Phe-Leu-Phe

We have shown previously that the fluorescence response to suboptimal concentrations of F-Met-Leu-Phe, but not to equivalent concentrations of C5a, can be effectively blocked by 10^{-5} M Boc-Phe-Leu-Phe-Leu-Phe (18). Boc-Phe-Leu-Phe-Leu-Phe is a competitive inhibitor of the binding of F-Met-Leu-Phe, thus antagonizing the functional responses which the synthetic peptide induces. Boc-Phe-Leu-Phe-Leu-Phe and other similar synthetic peptide antagonists are, however, without effect on C5a-induced functional responses (1, 22). We have extended these observations and studied the effects of various concentrations of F-Met-Leu-Phe and their modulation by Boc-Phe-Leu-Phe-Leu-Phe by measuring the fluorescence intensity decrease induced by different concentrations of F-Met-Leu-Phe in 3 min (a time long enough for the completion of the effect) and normalizing it to that produced by 10^{-8} M F-Met-Leu-Phe. Similarly, one can normalize the fluorescence changes observed in the presence and absence of Boc-Phe-Leu-Phe-Leu-Phe to that of the control condition with no inhibitor present. The results of a number of such experiments are summarized in Fig. 3. Small but significant fluorescence changes are detectable at concentrations of F-Met-Leu-Phe as low as 10^{-10} M. Close to maximal effects of F-Met-Leu-Phe on the fluorescence of cell-associated chlorotetracycline are observed with 10^{-8} M F-Met-Leu-Phe. The concentrations of F-Met-Leu-Phe necessary to elicit fluorescence changes are thus similar, if not identical, to those required to activate the various chemotactic factor-dependent functions of neutrophils. Fig. 3 shows,

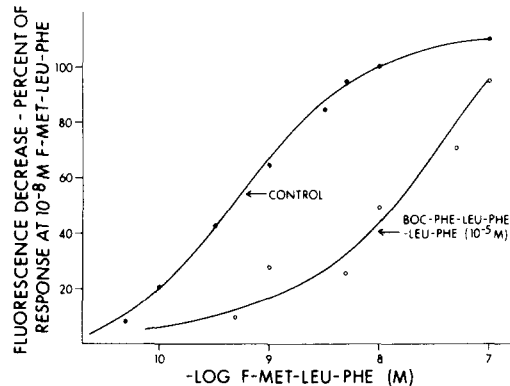


FIGURE 3 Dose-response curve for the F-Met-Leu-Phe-induced decrease of the fluorescence of cell-associated chlorotetracycline and the effect of Boc-Phe-Leu-Phe-Leu-Phe. The cell suspensions were pre-equilibrated with chlorotetracycline (100 M), washed, and resuspended all in the presence of 0.5 mM Ca^{2+} . F-Met-Leu-Phe was added at the concentrations indicated during the slow phase of the intrinsic fluorescence decrease. Variability among individual sample points was <15%. Each point represents the mean of at least three determinations.

in addition, that Boc-Phe-Leu-Phe-Leu-Phe can inhibit the effect of low, but not high, concentrations of F-Met-Leu-Phe on the fluorescence of chlorotetracycline. In other words, Boc-Phe-Leu-Phe-Leu-Phe appears to behave like a competitive inhibitor of the F-Met-Leu-Phe-induced fluorescence changes. Assuming competitive inhibition, a dissociation constant, K_d , for Boc-Phe-Leu-Phe-Leu-Phe can be calculated from the shift of the dose-response curves shown in Fig. 3 (2). The K_d so calculated is 4×10^{-7} M and is in good agreement with the values which were previously determined from binding and biological assays (reference 1 and unpublished data) and which range from 2.5 to 8×10^{-7} M.

Effect of the Chemotactic Factors on the Spectral Characteristics of Cell-Associated Chlorotetracycline

Mg^{2+} as well as Ca^{2+} chelates with and increases the fluorescence of membrane-bound chlorotetracycline. Thus, the changes in chlorotetracycline fluorescence could be due to a decrease in calcium binding or of magnesium binding or both or to a simple loss of chlorotetracycline from the membranes. To differentiate among these possibilities, advantage was taken of the fact that magnesium-chlorotetracycline exhibits excitation and emission

maxima at wavelengths that are ~ 10 nm shorter than the wavelengths of the respective maxima of the calcium-chlorotetracycline complex (8). In these experiments, excitation and emission spectra of cell-associated chlorotetracycline were obtained before and after the addition of 10^{-8} M F-Met-Leu-Phe to neutrophils preloaded with the chelate probe. The results of these experiments clearly show a small, but significant, shift towards shorter wavelengths of the excitation and emission maxima of chlorotetracycline upon the addition of the chemotactic factor (Fig. 4). The control spectra exhibit the expected maxima intermediate between those that would be obtained from the purified calcium and magnesium chelates of this probe (5, 6, 8). The fluorescent signal from untreated neutrophils thus originates from a mixture of the calcium and magnesium complexes of chlorotetracycline. The shift towards shorter wavelength of the spectral maxima of chlorotetracycline which is induced by F-Met-Leu-Phe indicates that after stimulation a larger proportion of the fluorescent signal originates from the magnesium complex of chlorotetracycline and thus that some of the membrane calcium must have been displaced.

Relationship between the Pools of Calcium Displaced by F-Met-Leu-Phe and C5a

Additional insight into the mechanism of action of F-Met-Leu-Phe and C5a was obtained by studying the relationship between the pools of calcium which are released by F-Met-Leu-Phe and by C5a. The results are summarized in Figs. 5 and 6. The

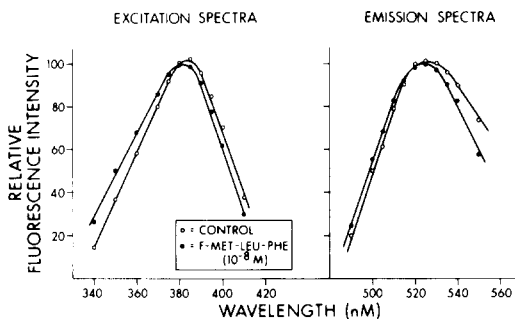


FIGURE 4 Spectral characteristics of the fluorescence of cell-associated chlorotetracycline before and after the addition of F-Met-Leu-Phe. The experimental manipulations were as in Fig. 3. The spectra were recorded during the slow phase of the intrinsic fluorescence decrease and 3 min after the addition of 10^{-8} M F-Met-Leu-Phe. The emission and excitation wavelengths were set at 520 and 390 nm for the excitation and emission spectra, respectively.

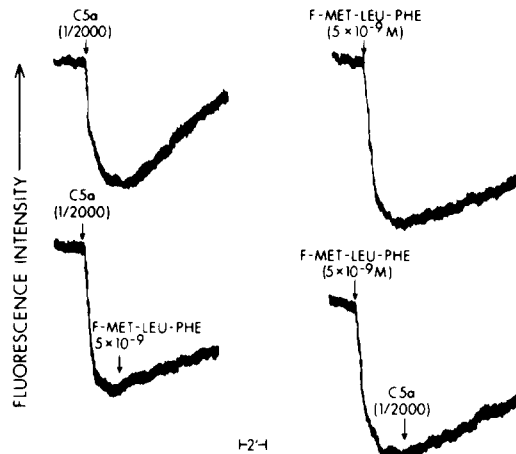


FIGURE 5 Sequential addition of F-Met-Leu-Phe and C5a to neutrophils during chlorotetracycline uptake experiments. Experimental conditions as in Fig. 1. The chemotactic factors were added at the steady-state of the chlorotetracycline uptake curve.

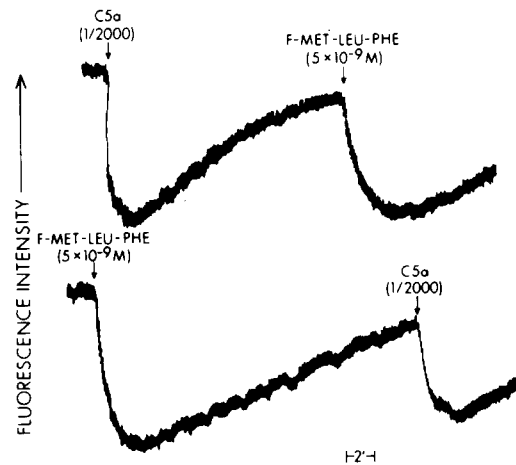


FIGURE 6 Sequential addition of F-Met-Leu-Phe and C5a to neutrophils during chlorotetracycline uptake experiments. Conditions as in Figs. 1 and 5. The chemotactic factors were added at the steady-state of the chlorotetracycline uptake curve.

addition of either of the stimuli at the steady-state of chlorotetracycline uptake results in a transient decrease in the fluorescence signal. The addition of the second chemotactic factor during the trough of the fluorescence changes does not cause any additional decrease of the fluorescence signal (Fig. 5). As the original stimulation only displaces $\sim 20\%$ of the membrane-bound chlorotetracycline, these results make it very unlikely that the recovery of the fluorescence is solely due to the reestablish-

ment of the concentration of chlorotetracycline in the membrane(s). However, if one waits until the fluorescence steady-state is restored before adding to the cells the second stimuli, the cells now respond nearly as efficiently as control, nonstimulated cells (Fig. 6). In other words, the cells have recovered from the initial stimulation even though the original stimulus is still present in the incubation medium. These results strongly suggest that, although initiated by their binding to different receptors, the actions of F-Met-Leu-Phe and C5a are mediated, at least in part, through the release of a common pool of membrane calcium.

Effect of Cytochalasin B on the Chemotactic Factor-Induced Fluorescence Changes

The various drastic effects of cytochalasin B on neutrophils exposed to chemotactic factors have been described in detail previously and include lysosomal enzyme release and, perhaps more relevant to this study, large enhancements of the chemotactic factor-induced fluxes of Na^+ and Ca^{2+} . The effect of cytochalasin B on the fluorescence changes caused by F-Met-Leu-Phe was thus examined next, and the results obtained are described in Fig. 7. These experiments were carried out at the steady-state of chlorotetracycline uptake. Cytochalasin B, by itself, was found not to significantly affect the steady-state level of fluorescence or the initial decrease in fluorescence caused by F-Met-Leu-Phe. This is to be contrasted to the effects of cytochalasin B on the F-Met-Leu-

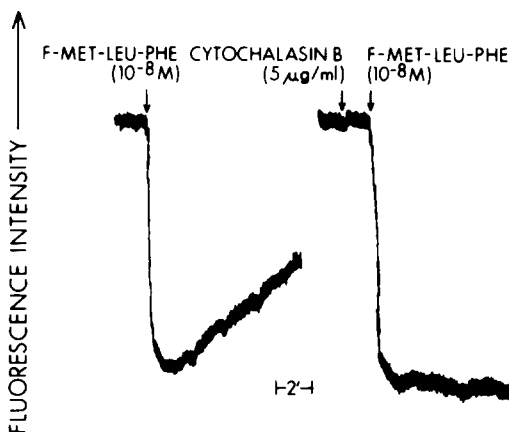


FIGURE 7 Effect of cytochalasin B on the fluorescence decrease caused by F-Met-Leu-Phe during chlorotetracycline uptake experiments. Conditions as in Fig. 1. F-Met-Leu-Phe and/or cytochalasin B were added at the steady-state of the chlorotetracycline curve.

Phe-induced cation fluxes (18, 20). On the other hand, in the presence of cytochalasin B, the recovery of fluorescence that follows the initial decrease of fluorescence is inhibited. It is possible that this effect is a direct result of an interaction of cytochalasin B with the mechanism(s) responsible for the binding of membrane calcium. It is impossible at this time, however, to rule out the possibility that a nonspecific damage to the cells due to the degranulation is responsible for the inhibition of the recovery of the fluorescence.

DISCUSSION

The dependence of the fluorescence of chlorotetracycline on divalent cations has been amply documented (4, 6, 8, 15). Because of its hydrophobicity, chlorotetracycline is an extremely useful tool for the direct investigation of the state of calcium bound to various membranes. It gives results that could only be indirectly guessed with previously used techniques. These properties have been utilized to study the effects of various stimuli on the pancreatic acinar (7-9) and β (31) cells.

As previously reported (18), F-Met-Leu-Phe and C5a decrease the fluorescence of cell-associated chlorotetracycline. Two lines of experimental evidence support the notion that the chemotactic factor-induced fluorescence changes are caused, at least partially, by a release of membrane calcium. Fig. 5 shows that, after stimulation by chemotactic factors, the spectral maxima of chlorotetracycline excitation and emission are shifted towards shorter wavelengths. In other words, the spectral characteristics of the fluorescence signal resemble those of the Mg^{2+} chelate of chlorotetracycline after stimulation with F-Met-Leu-Phe more than they did before the addition of the chemotactic factor. As the initial signal originates from a mixture of both of the Mg^{2+} and the Ca^{2+} chelates of chlorotetracycline, it follows that some of the membrane calcium must have been displaced upon stimulation. Essentially similar results and interpretations have recently been presented in the pancreatic acinar cells (8).

The second type of evidence comes from polarization experiments performed in model solvents and in biological membranes (6, 32). These studies eliminate the possibility that stronger Ca^{2+} -ligand interaction could account for the stimuli-induced fluorescence decrease that has been observed here and elsewhere (6, 32). Taken together, these results indicate that the most likely explanation for the decrease in fluorescence of cell-associated chlor-

otetracycline which is induced by chemotactic factors is that the concentration of calcium in the microenvironment probed by chlorotetracycline is reduced upon stimulation.

The concentrations of chemotactic factors or antagonists required, respectively, to either elicit the decreases in fluorescence by the chemotactic factors or to inhibit those induced by the chemotactic agents are similar if not identical to those required to induce or inhibit the various functions of the neutrophils (27–30). These findings suggest that the changes in fluorescence induced by the synthetic chemotactic peptide occur through the latter's ability to interact with the same receptor responsible for the biological functions. This conclusion is further strengthened by the finding that Boc-Phe-Leu-Phe-Leu-Phe, a competitive antagonist of F-Met-Leu-Phe and similar synthetic peptides, is without effect on the fluorescence changes caused by C5a, a chemotactic factor structurally different from F-Met-Leu-Phe and which binds to different receptors (22). Moreover, Boc-Phe-Leu-Phe-Leu-Phe, by itself, is without effect on the fluorescence of the cells. This further shows that the fluorescence changes caused by F-Met-Leu-Phe are not due to a generalized nonspecific membrane disturbance, since the competitive antagonist is a peptide of similar hydrophobicity and size as the chemotactic factor. Functional activation of the plasma membrane receptors for F-Met-Leu-Phe is thus required for the fluorescence changes to occur.

The kinetics of the fluorescence changes are rapid enough to be consistent with a precursor role in even the most rapidly occurring of the neutrophils' functions, namely, lysosomal enzyme release, superoxide generation, and stimulated cation fluxes. The lack of effect of cytochalasin B on the F-Met-Leu-Phe-induced fluorescence changes is consistent with this interpretation. Experimental difficulties such as unknown mixing times, however, have made it impossible so far to accurately estimate the time of onset of the fluorescence changes. It is clear, however, that these times are of the order of seconds, or less.

The two chemotactic factors tested in the present study thus induce a release of membrane calcium in a manner which satisfies all of the necessary specificity requirements for being of functional significance in the mechanism of activation of the neutrophils. In addition, these results strongly suggest that, although mediated by their binding to different receptors, the actions of F-Met-Leu-Phe

and C5a are mediated at least in part through the release of a common pool of membrane calcium. This calcium is probably released to the inside of the cells since the divalent cation chelators EDTA and EGTA do not affect the chemotactic factor-induced release of calcium.

Since relatively short times are required for even low concentrations of chlorotetracycline to reach the compartment that is responsive to chemotactic factors, and since the cytoplasm of the neutrophil is characteristically devoid of endoplasmic reticulum and mitochondria, it appears very likely that the plasma membrane may be the membranous compartment that is being monitored in this study. Additional microscopical, pharmacological, and biochemical evidence, however, is required for the positive localization of the neutrophils' calcium storage pools.

The chemotactic factor-induced release of membrane calcium correlates well with a number of previously published observations concerning neutrophil physiology. For example, Gallin et al. (14) have described pyroantimonate deposits, presumably due to calcium, at the leading edge of cells oriented in a chemotactic gradient and more specifically at points of attachments of the cells with the filters. In addition, the enrichment in microfilaments in the pseudopods of locomoting and phagocytosing (26) neutrophils has been recognized for a long time. A local release of calcium from the plasma membrane of the neutrophils provides a simple framework within which these, and other, various findings can be accommodated.

In summary, then, the results of this investigation provide new and direct evidence for the involvement of intracellular calcium in the initiation and perhaps regulation of some of the neutrophils' functions which are activated by chemotactic factors. Experimental difficulties preclude for the moment the accurate quantification of the fluorescence changes. It is thus unclear at present whether the amount of calcium which is released is of a sufficient magnitude to activate the neutrophils by itself or whether it acts only as a trigger for some subsequent amplification mechanism. In either case, the chemotactic factor-induced release of calcium, the initial characterization of which has been described here and elsewhere (18), will most probably play a central role in any future description of the various events involved in the activation of the neutrophils.

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