American Journal of Pathology, Vol. 129, No. 1, October 1987

ROUS-WHIPPLE AWARD LECTURE

The Formylpeptide Receptor of the Neutrophil
A Search and Conserve Operation

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THE NEUTROPHIL, because of its intrinsic interest and importance in pathology, infectious disease, and various fields of medicine and biology, is an object of intense and diverse investigation by a large number of research workers. The results of their investigations have raised the cell to the forefront of studies leading to our present large and increasing understanding of the details of the several general patterns of cell activation and stimulus/response coupling that are being rapidly delineated at the molecular level. Aside from the investigators themselves, a not inconsiderable reason for the progress made in the study of neutrophil activation has been the easy, commercial availability of a number of inexpensive, pure, structurally well-defined oligopeptide chemotactic factors, the so-called formylpeptide chemotactic factors, to serve as stimuli. The story of the formylpeptide chemotactic factors and their neutrophil receptor is a cautionary tale, illustrating the primacy of an idea (as has been pointed out, "In the beginning was the word. . . ") and the importance to scientific progress of readily available tools. It is also a peg on which to hang a summary of some of the extraordinary progress that has been and is still being made in elucidating the biochemical mechanisms of stimulated neutrophil functions.

It is the story of these formylpeptide chemotactic factors and some of the progress in our understanding of neutrophil activation that they largely have made possible that I wish to review here. In what follows, after first recounting, in a brief fashion, how the formyl peptides came to be recognized as chemotactic factors, I shall describe the present state of our knowledge of the specificity of the neutrophil formylpeptide receptor and the biological significance of the formylpeptides as chemotactic factors. From there, I shall very briefly review some of the transductional events consequent to the binding of the chemotactic peptide to its receptor, particularly the role and the nature of the GTP binding protein that couples the signal induced by the peptide to the early enzymatic events resulting in neutrophil functions.

Recognition of the Chemotactic Activity of the Formylpeptides

The recognition of the chemotactic activity of the formylpeptides was the result of the brilliant, imaginative insight of Dr. Elliot Schiffmann, currently at the National Cancer Institute. Ward and Lepow previously had shown that culture filtrates from a number of bacterial species were chemotactic.[1] Schiffmann and I joined together to isolate and purify chemotactic factors from *Escherichia coli* culture filtrates and established that they were small peptides with blocked amino groups.[2] However, Schiffmann did more, asking himself the general question "Why are bacterial components chemotactic for eukaryotic cells?" His answer postulated that it was because the eukaryotic cells were recognizing substances that were non-eukaryotic, typically prokaryotic cell products. One very typical prokaryote product is the formylmethionyl peptides, since prokaryotes initiate protein synthesis with formyl methionine, whereas

Supported in part by NIH Grant AI 09648.

Presented in part as the Rous–Whipple Award Lecture at the annual meeting of the American Association of Pathologists, Washington, DC, March 31, 1987.

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eukaryotes utilize nonacylated methionine. In testing this hypothesis, Schiffmann demonstrated that formylmethionine and simple formylmethionyl dipeptides and a few tripeptides were indeed weakly but significantly chemotactic for neutrophils.[3] (This quite unexpected result demonstrated once again that by taking thought a man can add more than one cubit to his scientific stature.) Richard Freer and I, with Schiffmann's permission, immediately took advantage of Schiffmann's results to begin the study of the structural requirements for activity of the formylpeptides with neutrophils. In our initial study, 24 peptides were synthesized; and with the aid of Henry Showell, we showed the very great sensitivity of the chemotactic and the granule releasing activities of the 24 peptides to even small changes in structure.[4] This latter result led to the suggestion that the formyl peptides acted by binding to a receptor on the neutrophil surface. This suggestion was shown to be correct when radiolabeled formylpeptides were found to bind with rabbit and with human neutrophils in a saturable and reversible fashion with a rank order of binding that was quantitatively the same as the rank order of their biologic activity.[5,6] The receptor has been isolated by several laboratories and shown to be a 55,000–75,000 molecular weight glycoprotein.[7,8]

As with other neutrophil chemoattractants, the term "formylpeptide chemotactic factors" does less than full justice to their versatility. The early study showed that they were able not only to induce chemotaxis but also to cause granule enzyme secretion; and subsequent investigations demonstrated that they could cause the neutrophil to undergo the "respiratory burst," including the generation of superoxide, to aggregate, and if placed on particles, to phagocytize the particle (reviewed by Becker[9]). Nevertheless, as a matter of convenience, they will be referred to as formylpeptide chemotactic factors although the term "formylpeptide pan-activators" is more accurate. It also should be pointed out that in addition to neutrophils, the formyl peptides activate eosinophils and basophils as well as macrophages/monocytes and have other biologic activities. Because the great bulk of the work has been done with neutrophils, I shall refer, in what follows, only to their actions on these cells.

Specificity of the Neutrophil Formylpeptide Receptor

The Hebrew *Talmud* assures us that "The work of the righteous is done by others."* The list of collaborators on the work defining the specificity of the neutrophil formylpeptide receptor, seen in Table 1, is the only sort of claim to righteousness that I can make; and I have a small, nagging suspicion that this may not be what the *Talmud* meant. In this work, I wish to acknowledge not only the central role of Dr. Richard J. Freer and Mr. Henry Showell but also the important contributions of Dr. Claudio Toniolo of the University of Padua and Dr. Padramaham Balaram of the India Institute of Science.

A practical result of the initial studies was the synthesis and demonstration of the biologic activity of Nα-formyl methionylleucylphenylalanine, f-Met-Leu-Phe-OH, or FMLP, as it also called in that "furor acronymicus" which so afflicts our common understanding. The structure of f-Met-Leu-Phe-OH is shown in Figure 1. It is highly active biologically with an ED_{50} (the concentration giving 50% of the maximal activity) for chemotaxis of rabbit neutrophils of 7×10^{-11}M and 2.5×10^{-10}M for inducing lysozyme secretion.[9] For many years it was the most active of the formylpeptides and is still the most widely employed. Using f-Met-leu-Phe-OH as the prototypical tripeptide, the initial structure–activity studies were extended by systematically varying each element of its structure, by modifying or eliminating the formyl group, and by varying the length and nature of the side chains of the methionyl, leucyl, and phenylalanyl residues.[10,11] One clear-cut result of the earlier studies was the demonstration of the necessity for the N-formyl group for both binding and activity,[10] hence the name "formylpeptide chemotactic factors." Table 2 shows that removal of the formyl group leads to a 2800-fold drop in activity. The activity cannot be restored by substitution of an N-acetyl group or removal of the terminal amino group,[10] and unpublished work also demonstrates that if the carbonyl group of the N-formyl residue is reduced to an alcohol, activity is depressed 570-fold.

The results of the study of over 100 peptides are summarized in the model of the binding of f-Met-Leu-Phe-OH to the neutrophil receptor seen in Figure 2. In this model, there are five major areas of interaction between peptides and receptor[11]:

Table 1—Collaborators: The Specificity of the Formyl Peptide Receptor

Dr. Richard J. Freer	Medical Collegte of Virginia, Richmond, Virginia
Dr. Claudio Toniolo	University of Padua, Padua, Italy
Dr. Padmanabhan Balaram	India Institute of Science, Bangalore, India
Dr. Jay Glasel	University of Connecticut Health Center, Farmington, Connecticut
Mr. Henry Showell	Pfizer Central Research, Groton, Connecticut

*My thanks to Dr. Nathan J. Zvaifler for bringing this quotation to my attention.

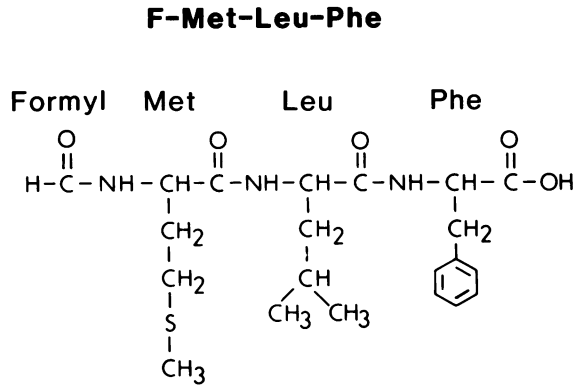

Figure 1—The structure of Nα-formylmethionylleucylphenylalanine (f-Met-Leu-Phe-OH, FMLP). The amino acids in all peptides discussed are of the L configuration.

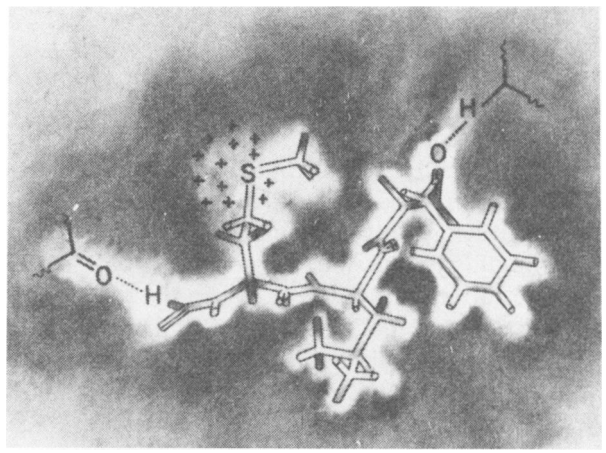

Figure 2—Model of the interaction f-Met-Leu-Phe-OH with the receptor in rabbit neutrophils. Reproduced from Freer et al[11] by permission of the American Chemical Society.

1. In view of the specificity of the requirement of an N-formyl group for high or even moderate activity, we postulate hydrogen bonding of the formyl proton to a critical area on the receptor containing a hydrogen bond receptor. Supporting this hypothesis is the finding that deutero-formyl-methionyl-leucylphenylalanine (^{2}DCO-Met-Leu-Phe-OH) in which deuterium is substituted for the hydrogen of the formyl group is 3–4-fold more active than CHO Met-Phe-OH itself.[12]) This is in accord with the well-known enhancement of hydrogen bonding resulting when deuterium is substituted for hydrogen, as seen with some enzyme–substrate interactions.[13]
2. The methionyl side chain fits into a hydrophobic pocket of limited depth, because derivatives with side chain lengths longer than 4 carbon atoms show no further increase in activity. Moreover, there appears to be a tight fit of the pocket in the vicinity of the δ carbon, because branching at this point results in loss of activity, whereas in the area of the β carbon the fit appears to be much looser.

Table 2—Requirement for the N α-Formyl Group

	Lysozyme release	
	$ED_{50} \pm SEM$ (M)	Relative potency
HCO-Met-Leu-Phe-OH	$(3.2 \pm 0.8) \times 10^{-10}$	
CH_3CO-Met-Leu-Phe-OH	$(1.4 \pm 0.2) \times 10^{-6}$	4400*
Met-Leu-Phe-OH	$(8.9 \pm 1.4) \times 10^{-7}$	2800*
desamino Met-Leu-Phe-OH	$(1.1 \pm 0.3) \times 10^{-6}$	3400*
HCO-Nle-Leu-Phe-OH	$(9.2 \pm 0.1) \times 10^{-10}$†	
HCOH-Nle-Leu-Phe-OH	$(5.3 \times 0.6) \times 10^{-7}$†	588‡

*Fall in activity relative to HCO Met-Leu-Phe-OH; taken from Freer et al.[10]
†Different experiment, ED_{50}s corrected for different ED_{50}s of HCO Met-Leu-Phe-OH in the two groups of experiments.
‡Fall in activity relative to HCO Nle-Leu-Phe-OH.

We also postulated a discrete area of positive charge in the receptor complementary to the relatively electron-rich sulfur atom to account for the approximately 5-fold greater activity of f-Met-Leu-Phe-OH, compared with the non-sulfur-containing counterpart, formylnorleucylleucylphenylalanine (f-Nle-Leu-Phe-OH).[10]

3. The leucyl side chain also fits into a hydrophobic pocket. The fit around the β carbon seems to be relatively loose, there being no difference in activity between peptides containing norvaline, valine, or isoleucine. That the pocket is large relative to the leucyl group is suggested by the finding that formylmethionyl α aminocyclohexylphenylalanine methyl ester (f-Met-Acc^6 Phe-OMe) is as active or somewhat more active than the corresponding f-Met-Leu-Phe-OMe.[14]
4. A third hydrophobic pocket accommodates the phenylalanine side chain. The fit of the pocket to the aromatic ring side chain seems to be very tight. Alkyl substituents uniformly give less activity, substitution of a chloro group to form the p-chlorophenylalanyl residue leads to a 40 fold[15] drop in activity, and insertion of a p-hydroxyl to form a tyrosyl residue leads to an almost 120-fold decrease.[11]
5. The carbonyl of the phenylalamine is required. As seen in Table 3, removal of the carboxyl group, as in f-Met-Leu-β-phenylethylamine, decreases activity 62-fold relative to f-Met-Leu-Phe-OH; reducing the carboxy group to the corresponding alcohol decreases activity 22-fold, whereas the benzyl ester of f-Met-Leu-Phe-OH is 62 times more active than the parent compound. This last result is characteristic of the greater or lesser in-

Table 3—Requirement for a Carbonyl Group in Position 3 Analogs

	ED_{50} for lysozyme release (M)	Potency ratio
CHO-Met-Leu-Phe-OH	$(3.2 \pm 0.8) \times 10^{-10}$	
CHO-Met-Leu-Pea*	$(2.0 \pm 0.3) \times 10^{-8}$	0.016§
CHO-Met-Leu-Phe-OBzl†	$(4.6 \pm 1.2) \times 10^{-11}$	12§
CHO-Nle-Leu-Phe-OL‡	$(1.7 \pm 0.70) \times 10^{-8}$	0.046‖

*Pea, phenylethylamine.
†BZl, benzyl.
‡Phe-OL, phenylalanylol.
§Potency ratio versus CHO-Met-Leu-Phe-OH.
‖Potency ratio versus CHO-Nle-Leu-Phe-OH.
All data except for CHO-Nle-Leu-Phe-OL are taken from Freer et al.[11]

creases in activity given by esters or amides of the tripeptide.[16,17] Thus, only those carboxyl modified analogs that retain the carbonyl function of the phenylalanine display high biologic activity. We therefore postulate that the phenylalanyl carbonyl interacts with a critical area on the receptor, probably as an acceptor for a hydrogen bond.[11]

The model, as pictured, is obviously incomplete; even more, it describes, at best, only the interactions with that portion of the receptor that accommodates a tripeptide. There is evidence, however, that the receptor is, at least, large enough to fit a tetrapeptide. This is suggested by the 10-fold or greater increase in activity of the benzyl esters and benzylamide of the tripeptides[11] and the greater or lesser increases of activity of the long chain esters and amides of f-Met-Leu-Phe-OH.[16] Moreover, as one increases the number of methionyl residues in the $f(Met)_n$-OMe series of homoligopeptides, the activity increases correspondingly, up to the tetrapeptide; however, the pentapeptide and hexapeptide show no further increment.[17] Table 4, showing the effects on activity of adding an additional amino acid to f-Met-Leu-Phe-OH, confirms and extends this last finding. The tetrapeptides f-Met-Leu-Phe-Ile-OH and f-Met-Leu-Phe-Phe-OH are, respectively, 12 and 20 times more active than the parent tripeptide, demonstrating that here also the tetrapeptides are distinctly more active than the corresponding tripeptides. The 5-fold or greater *decrease* in activity of f-Met-Leu-Phe-Lys, containing the terminal polar lysine group, suggests that as in the other positions, the pocket for fourth position amino acids is also hydrophobic. The fact that there is little or no difference in the activity of f-Met-Le-Phe-Ile-OH, compared with f-Met-Leu-Phe-Phe-OH, suggests that unlike the third position, an aromatic group in the fourth position fits loosely into the corresponding hydrophobic pocket. However, to really define the nature of this subsite of the receptor, a systematic investigation is required and planned. That there might be room in the receptor to accommodate at least a portion of a fifth peptide is suggested by the finding of Niedel and co-workers that f-Met-Leu-Phe-Met-Tyr-OH is approximately 4-fold more active chemotactically than f-Met-Leu-Phe-Met-OH, the latter being approximately 10-fold more active than f-Met-Leu-Phe-OH.[18]

Table 4—The Effect of Adding an Amino Acid to the Carboxyl Terminus of CHO-Met-Leu-Phe-OH

	ED_{50} for lysozyme release (M)	Potency ratio
CHO-Met-Leu-Phe-OH	$(5.5 \pm 2.2) \times 10^{-10}$*	
CHO-Met-Leu-Phe-Ile-OH	$(4.6 \pm 0.6) \times 10^{-11}$*	12
CHO-Met-Leu-Phe-Phe-OH	$(2.7 \pm 0.9) \times 10^{-11}$*	20
CHO-Met-Leu-Phe-Lys-OH	$(2.9 \pm 0.9) \times 10^{-9}$†	0.19

In all cases the potency ratio is versus CHO-Met-Leu-Phe-OH.
*Taken from Freer et al.[11]
†Taken from Freer et al[10]; ED_{50} corrected for the different ED_{50}s of HCO-Met-Leu-Phe-OH in the two groups of experiments.

Despite the satisfactory, albeit incomplete, progress in deciphering the nature of the interactions of the formylpeptide with its neutrophil receptor, we are still uncertain as to the conformation of the peptide in the receptor. Based on a nuclear magnetic resonance (NMR) investigation by Dr. Glasel of f-Met-Leu-Phe-OH in solution,[19] we have suggested that the peptide exists on the receptor in an extended β-pleated sheet (antiparallel) conformation.[11] This has been supported by Dr. Toniolo and his co-workers, who studied a series of biologically active formylhomoligopeptides and related their conformation in the solid and liquid states as revealed by infra-red spectophometry and circular dichroiism to their biologic activity.[17,20] A difficulty with this approach, which we fully realized from the beginning, is that the conformation of the peptide may be quite different in the receptor to that found in solution or the solid state. Point to this caveat is given by the finding that analogs of f-Met-Leu-Phe-OH which are conformationally restricted through steric constraints about the side chains to favor a folded backbone conformation are biologically highly active[21] in fact, one of them in which aminocyclohexane carboxylic acid is substituted for the leucine of f-Met-Leu-Phe-OMe is as, or even more, active than f-Met-Leu-Phe-OMe.[14]

In an attempt to obtain a more definitive answer to the question of the conformation of the peptide in the active site we are starting studies using computer simulation. In very preliminary work, Dr. Freer, in collaboration with Dr. Garland Marshall, employing the SYBYL software package from Tripos Associates, has determined the α helical and the extended conforma-

tion of the analog N-formyl-Met-Aib-NαCH$_3$-Phe-OH, where Aib is α-aminoisobutyric acid. This analog was designed on the basis of the high activity of both f-Met-Aib-Phe-OH[21] and unpublished work with Dr. Freer showing the high activity of f-Met-Leu-NαCH$_3$-Phe-OH). The two conformations were obtained with an energy minimization program. The α helical conformation calculates to −9.6 kcal; the computed energy of the extended conformation, similar to that initially proposed by us to exist on the receptor, is −5.6 kcal. Thus, energetically speaking, the extended conformation is not preferred and on the basis of our model, should yield a much reduced activity. A high level would, however, support a folded conformation as the receptor-bound form of the molecule. This analog is currently being prepared and will be tested.

The possibility that the interaction of the formylpeptide with its neutrophil receptor involves an "induced fit" is one that cannot be ignored and assumes special relevance in view of the work of Edmundson and Ely.[22] Marasco and I showed, testing approximately 40 analogs of f-Met-Leu-Phe-OH, that the structural requirements for binding of the analogs to polyclonal antibodies against f-Met-Leu-Phe-OH are remarkably similar to those required for activity against neutrophils.[23] Edmundson and Ely found that the structural requirements for binding of formylpeptides to the hydrophobic antibodylike binding site cavity in crystals of the Bence–Jones protein Mcg are also remarkably similar to that found for the binding of the same compounds to the neutrophil formylpeptide receptor. Using X-ray crystallographic analysis, they demonstrated that the fit of the ligand to the binding site was the result of the moving of side chains and polypeptide segments making up the binding cavity of the protein with a corresponding folding and alteration of the peptide bond geometry of the ligand to improve the lock and key effect. That similar sliding, slithering, and bending are involved in the fit of the formyl peptides to the neutrophil is a possibility that can be neither denied nor overlooked. Unfortunately, similar x-ray crystallographic studies of the neutrophil receptor seem a distant dream.

Physiologic Function of the Formylpeptide Reactivity With Neutrophils

Given the high activity of these formylpeptides and the existence of specific receptor for them on leukocytes, a natural question is, "Do they have a physiological function?" As John Keats noted, "A question is the best beacon towards a little speculation." Schiffmann's original idea was that the formylpeptides were models for bacterial chemotactic factors. The discovery by Marasco and his co-workers, almost 10 years after the synthesis and testing of f-Met-Leu-Phe-OH, that this peptide is the most active chemotactic component of *E coli* culture filtrates showed that the formylpeptides are not only models of, but in fact are, bacterial chemotactic factors.[24] Even without this confirmation, Schiffmann's initial idea led to the assumption that the formylpeptide receptors on neutrophils and macrophages are adapted to respond to signals set up by bacteria themselves and thus are of importance in bacterial infections. Widespread as is the acceptance of this assumption, it is probably wrong. Dog,[25] bovine,[26] and pig[27] polymorphonuclear leukocytes do not bind f-Met-Leu-Phe-OH, and the polymorphonuclear leukocytes (PMNs) from same species are not chemotactically responsive to the peptide nor are the granulocytes from the cat[28] or horse,[29] even though the last secrete granule enzymes in response to f-Met-Leu-Phe-OH. These findings make it unlikely that the formylpeptides play any general role in the response of phagocytic leukocytes to bacterial infection.

An alternative hypothesis, which I expressed several years ago,[30] is that the major physiologic site of the formylpeptide receptor is not on the leukocyte, but on some other cell or tissue and that its natural agonist is an endogenous peptide, polypeptide, or protein. The observations that f-Met-Phe-Leu-OH induces receptor-mediated chemotaxis in human[31] and bull spermatozoa,[32] the spasmogenic activity of formylpeptides on the pig guinea ileum,[33] and the constriction they induce in the isolated guinea pig trachea[34] or lung parenchyma[35,36] all lend credence to this idea. These findings imply that we must look for an endogenous ligand acting with a formylpeptide receptor on nonleukocyte cells or tissues as the physiologically important system. What this system is or even whether it exists only future work will tell.

GTP Binding Regulatory Proteins and the Effects of Pertussis Toxin in Neutrophil Function

In the study of the transductional events consequent to the reaction of specific neutrophil receptors with their specific ligands, the formylpeptides, and particularly f-Met-Leu-Phe-OH, have been the most widely used chemotactic factor stimulus. Table 5 names the numerous individuals with whom I have collaborated in those aspects of the studies in which I have been involved.

Stimulation of specific receptors with chemotactic factors causes neutrophils to undergo a large number

Table 5—Collaborators: The Transduction of the Formylpeptide

Mr. Henry J. Showell
Dr. Ramadan Sha'afi
Dr. Paul Naccache
Dr. Yasunori Kanaho
Dr. John Kermode
Dr. William Mackin
Dr. Barbara-Jean Bormann
Dr. Chi-Kuang Huang
Dr. Wayne Marasco
Dr. John Munoz
Mr. Scott Kennedy

of coordinated physiologic and biochemical responses which ultimately result in the various functional responses.[37] With the formylpeptides, as with other chemotactic factors, the coupling of the stimulus to most of these responses is through a GTP-binding regulatory protein. GTP binding regulatory proteins comprise a closely related family of proteins called "G" or "N" proteins. They couple receptor occupancy to activation of different effector enzyme systems and ion channels in a wide variety of cells stimulated by many different kinds of agonists and sensory stimuli.[38] The two GTP binding regulatory proteins, G_s and G_i, mediate the agonist-induced stimulation and inhibition, respectively, of adenylate cyclase. Another G protein, transducin, couples the photoactivation of rhodopsin in the rod outer segment of the retina to the activation of the cyclic GMP phosphodiesterase in the same site. The function of another G protein, G_o is uncertain. All these proteins are structurally very similar and have been highly purified and well characterized. They are heterotrimers consisting of distinct α subunits containing the enzymatic and effector interacting activity of the G protein. The α subunits are linked noncovalently to identical or very similar $\beta\gamma$ subunits. Certain bacterial toxins have been very helpful in elucidating the role of the G proteins; $G_s\alpha$ and the α subunit of transducin ($T\alpha$) are substrates for the adenosine diphosphate (ADP)-ribosylation catalyzed by cholera toxin and the α subunits of G_i, G_o, and transducin are substrates for the ADP-ribosylation catalyzed by pertussis toxin (the so-called pertussigen of Munoz, or the islet-activating protein of Ui). In the process, G_i and T are inactivated.

A role for the G proteins in the responses of neutrophils was first suggested by the reports of Koo et al[39] that guanyl nucleotides shift the affinity of the formylpeptide chemotactic receptors and of Hyslop and his co-workers that chemotactic factor stimulation increases the GTPase of neutrophil homogenates.[40] This latter finding has been confirmed by Okajima and Ui[41] and Feltner and co-workers,[42] and Sha'afi et al[43] have characterized the GTPase activity. In unpublished work, Drs. Yasunori Kanaho and John Kermode, while in my laboratory, have shown that the formylpeptides increase the GTPase activity of isolated plasma membranes by reacting with the same receptor that triggers granule enzyme release and, by inference, the other stimulated functions of the neutrophil. They tested four formylpeptides, differing over a 1000-fold range in their biologic activity, for their ability to increase the GTPase activity of isolated plasma membranes of rabbit neutrophils. The activity of the formylpeptides in increasing GTPase activity as measured by their ED_{50}s correlated almost exactly with the activity of the same peptides in inducing the secretion of β glucosaminidase, the correlation coefficient being 0.998. In addition, Dr. Kanaho showed that a specific antagonist of the formylpeptides, t-Boc-Phe-Leu-Phe-Leu-Phe-OH inhibits the GTPase-increasing activity of f-Met-Leu-Phe-OH as expected for a competitive inhibitor of formyl peptide binding to its receptor.

However, it is pertussis toxin which has served as a primary tool in the study of the G protein involved in the coupling of receptor–chemotactic factor binding to the stimulated neutrophil functions. The use by ourselves and a number of others of pertussis toxin to inhibit various neutrophil functions and biochemical and physiologic responses has permitted a clearer definition of both the role and nature of the G protein involved. For example, granule enzyme secretion by f-Met-Leu-Phe, C5a, leukotriene B_4,[44] and platelet activating factor[45] is reduced or abolished by pertussis toxin treatment in a time- and concentration-dependent manner. Results from a number of laboratories have shown that not only granule enzyme secretion but chemotaxis, superoxide (O_2^-) generation, aggregation, and capping induced by f-Met-Leu-Phe are also inhibited by pertussis toxin treatment (see reviews[37,46]). Okajima and Ui have demonstrated that the degree of inhibition of receptor-stimulated neutrophil function is proportional to the extent of ADP-ribosylation of the pertussis toxin substrate.[41] Interestingly, phagocytosis of opsonized particles[47,48] as well as the generation of O_2^- and release of myeloperoxidase by the same stimulus[47] are little inhibited, if at all, by pertussis toxin treatment. Whether it is the specific stimulus itself or its particulate nature which is responsible for the insensitivity to pertussis toxin is unknown. However, it clearly points up the fact that a pathway is available for these stimulated functions which does not involve, or involves only to a limited extent, the G protein required with soluble stimuli.

There is neither the space nor is this the occasion to give the detailed evidence accumulated by a large number of investigators that has established our

present picture of the biochemical sequences stimulated by the formylpeptides and other chemotactic factors and specifically the role of the G proteins in the sequences (see review[37]). For the present purposes, it suffices to briefly, inadequately, and incompletely summarize this present pictures as follows: Binding of receptor and chemotactic factor stimulates an exchange of GTP and GDP in the α subunit of the neutrophil G protein we call G_n. This leads to a dissociation of α_n from $\beta\gamma$ subunits in the G protein. The GTPα_n subunit activates a phosphatidyl inositol specific phospho-diesterase, phospholipase C. The GTP is broken down to GDP by the receptor induced stimulation of the GTPase activity of the α subunit to form the inactive GDPα_n which is then able to recombine with free $\beta\gamma$ subunit. The phospholipase C activated by the α_n subunit hydrolyzes phosphatidylinositol polyphosphates to form two second messengers, inositol 1,4,5-trisphosphate (IP_3) and sn-1, 2-diacylglycerol. The IP_3 releases Ca^{2+} from intracellular stores into the cytosol; the diacylglycerol activates the Ca^{2+} and phospholipid-dependent protein kinase protein kinase C.

Pertussis toxin ADP ribosylates G_n, inactivating it. The inactivated G_n is unable to mediate the receptor-coupled activation of phospholipase C. The results of pertussis toxin treatment not only provide critical evidence for the central role of G_n in these various reactions and show the importance of the G_n-mediated coupling of the receptor to phospholipase C activation, but also demonstrate the requirement of some or all of these reactions in the stimulated functions of the neutrophil.

The last subject to be discussed deals with the nature of the neutrophil G protein, which we have called G_n,[49] Gompertz has termed G_p,[50] and Snyderman and his group[51] and also Sha'afi[37] have termed G_c. Dr. Kanaho, while recently in my laboratory, has obtained evidence that the α subunit of G_n is biochemically distinct from those of other known G proteins that are pertussis toxin substrates. G_i, G_o, and transducin.[49] Incubation of neutrophil membranes with pertussis toxin and ^{32}P-NAD (nicotinamide adenine dinucleotide) causes the labeling of a single protein $G_{n\alpha}$. $G_{n\alpha}$ migrates on polyacrylamide gel electrophoresis, slightly faster than G_i but more slowly than $G_{o\alpha}$ or T_α. On this basis, $G_{n\alpha}$ is estimated to have a molecular weight of 40,000 daltons; this is slightly but significantly less than $G_{i\alpha}$, but greater than $G_{o\alpha}$ or T_α.[49] Isoelectric focussing clearly resolves rabbit $G_{n\alpha}$ from $G_{i\alpha}$ and $G_{0\alpha}$ and T_α purified from bovine retina. The isoelectric points of $G_{n\alpha}$, $G_{i\alpha}$, $G_{o\alpha}$ and T_α are approximately 5.7, 6.0, 5.5, and 5.9. Digestion by *Staphylococcus aureus* V8 protease of the four ^{32}P-ADP-ribosylated proteins gives patterns of peptide fragments on SDS-polyacrylamide gels that are slightly, but definitely and reproducibly, different from each other. In addition to these biochemical differences of G_n from the other known G protein substrates of pertussis toxin, Spiegel and his colleagues have presented evidence that G_n is immunochemically distinct from G_i,[52,53] and Snyderman and his co-workers have evidence that, like transducin, G_n is a substrate for not only pertussis toxin but cholera toxin as well.[51]

G_n may not be the only G protein involved in neutrophil response coupling. Gompertz, on the basis of the results of granule enzyme release from permeabilized neutrophils, has postulated that guanine nucleotides interact not only with G_n to activate phospholipase C but also with another G protein distal to the site of action of Ca^{2+} and protein kinase C. The later putative G protein appears not to be a pertussis toxin substrate.[50]

It is likely that G_n is not specific to the neutrophil. For example, the mast cell, when stimulated with 48/80, the platelet stimulated with thrombin, the insect salivary gland stimulated with serotonin, among other cell systems, also utilize a G protein which is a pertussis toxin substrate to mediate the activation of phospholipase C with all its attendant biochemical consequences. It is probable that, as in the neutrophil, the G protein involved is G_n or a closely similar protein. This remains for further work to prove. Given the rate of progress being made in our knowledge of the G proteins and their interactions, the proof one way or another should not be too long in coming.

Conclusions

In the past 10 years or so, our knowledge of the mechanisms of neutrophil activation, particularly by chemotactic factors, has come from almost total darkness to the distinct glimmerings of light. Real progress has been made in the definition of the nature and characteristics of the receptors involved not only with the formylpeptides, as this avowedly personal account has emphasized, but with other chemotactic factors as well. Numerous workers have joined together to define many of the changes, especially the early ones, that interaction of agonist and neutrophil induces: the participation and something of the nature of the mediatory G proteins, the alterations in fluxes of the cations Ca^{2+}, Na^+, H^+, and K^+, the changes in phospholipid metabolism, the production and action of intracellular mediators, or in the jargon, "second messengers," and the activation of protein kinases and resulting protein phosphorylation, among others. As splendid and exciting as this prog-

ress has been, the evidence, even in the areas mentioned, is very much incomplete and too often indirect, with conclusions largely or wholly dependent upon analogy with other systems or upon perturbation of intact cells with inhibitors or other agents of indeterminate specificity. We still have to isolate in a homogeneous state the components of the various pathways and then reconstitute their reactions to rigorously test their postulated interrelationships. Moreover, not only are there many aspects of what we think we know which have not been rigorously tested, but there are elements, probably important elements, even of these early events, that are missing and thus not part of our present picture. Even worse, the extent of our nescience and uncertainty with regard to these early events still cannot be compared to our almost total ignorance of how these early events are transmitted into the final functional responses of the neutrophil. However, having come so far, we only can have confidence that this darkness, too, shall be pierced by the light of knowledge.

References

1. Ward PA, Lepow IH, Newman LJ: Bacterial factors chemotactic for polymorphonuclear leukocytes. Am J Pathol 1968, 52:725–736
2. Schiffmann E, Showell HJ, Corcoran BA, Ward PA, Smith E, Becker EL: The isolation and partial characterization of neutrophil chemotactic factors from E. coli. J Immunol 1975, 114:1831–1837
3. Schiffmann E, Corcoran BA, Wahl SM: Formylmethionyl peptides as chemoattractants for leukocytes. Proc Natl Acad Sci USA 1975, 72:1059–1062
4. Showell HJ, Freer RJ, Zigmond SH, Schiffmann E, Aswanikumar S, Corcoran BA, Becker EL: The structure activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. J Exp Med 1976, 143:1154–1169
5. Aswanikumar S, Corcoran B, Schiffmann E, Day AR, Freer RJ, Showell HJ, Becker EL, Pert C: Demonstration of a receptor on rabbit neutrophils for chemotactic peptides. Biochem Biophys Res Commun 1977, 74:810–817
6. Williams LT, Snyderman R, Pike MC, Lefkowitz RJ: Specific receptor sites for chemotactic peptides on human polymorphonuclear leukocytes. Proc Natl Acad Sci USA 1977, 74:1204–1208
7. Niedel J: Detergent solubilization of the formylpeptide chemotactic receptor. J Biol Chem 1981, 256:9295–9299
8. Huang C-K: Partial purification and characterization of formylpeptide receptor from rabbit peritoneal neutrophils. J Leuk Biol 1986, 41:63–69
9. Becker EL: A multifunctional receptor on the neutrophil for synthetic, chemotactic oligopeptides. J Reticuloendothel Soc Suppl 1979, 83:179–186
10. Freer RJ, Day AR, Schiffmann E, Aswanikumar S, Showell HJ, Becker EL: Further studies on the structural requirements for synthetic peptide chemoattractants. Biochemistry 1980, 19:2404–2409
11. Freer RJ, Day AR, Muthukumaraswamy N, Pinon D, Wu A, Showell HJ, Becker EL: Formyl peptide chemoattractants: A model of the receptor in rabbit neutrophils. Biochemistry 1982, 21:257–263
12. Wu A, Muthukumaraswamy N, Freer RJ, Glasel JA, Mackin WM, Becker EL: Stable isotope containing peptides as probes of ligand receptor interactions: Deutero-formyl-methionyl-leucyl-phenylalanine. FEBS Lett 1983, 159:150–152
13. Aranyi P: Deuterium isotope effects on the rates of steroid-glucocorticoid receptor interactions. Eur J Biochem 1984, 138:89–92
14. Sukumar MP, Raz AP, Balaram P, Becker EL: A highly active chemotactic peptide analog incorporating the unusual residue 1-aminocyclohexanecarbhoxylic acid at position 2. Biochem Biophys Res Commun 1985, 128:339–344
15. Day AR, Radding JA, Freer RJ, Showell HJ, Becker EL, Schiffmann E, Corcoran B: Synthesis and binding characteristics of an intrinsically radio-labeled chemotactic acyl tripeptide: N^{α}-Formyl-norleucyl-leucyl-phenylalanine. FEBS Lett 1977, 77:291–294
16. Kraus JL: Studies on the structural requirements for synthetic formylpeptide chemoattractants. Pharmacol Res Commun 1980, 96:235–242
17. Toniolo C, Bonora GM, Showell HJ, Freer RJ, Becker EL: Structural requirements for formyl homo-oligopeptide chemoattractants. Biochemistry 1984, 23:698–704
18. Niedel J, Wilkinson S, Cuatrecasas P: Receptor mediated uptake and degradation of ^{125}I-chemotactic peptide by human neutrophils. J Biol Chem 1979, 254:10700–10706
19. Becker EL, Bleich HE, Day AR, Freer RJ, Glasel JA, Visitainer J: NMR conformational studies on the chemotactic tripeptide formyl-L-Met-L.Leu-L-Phe-OH: A small β sheet. Biochemistry 1979, 18:4656–4667
20. Bismara C, Bonora GM, Toniolo C, Becker EL, Freer RJ: Synthetic homooligomethionine chemoattractants: A conformation-activity study. Int J Peptide Protein Res 1985, 26:482–492
21. Iqbal M, Balaram P, Showell HJ, Freer RJ, Becker EL: Conformationally constrained chemotactic peptide analogs of high biological activity. FEBS Lett 1984, 259:1386–1393
22. Edmundson AB, Ely KR: Binding of N-formylated chemotactic peptides in crystals of Mcg light chain dimer: similarities with neutrophil receptors. Mol Immunol 1985, 22:463–475
23. Marasco WA, Showell HJ, Freer RJ, Becker EL: Anti f-Met-Leu-Phe similarities in fine specificity with the formyl peptide chemotaxis receptor of the neutrophil. J Immunol 1982, 128:956–962
24. Marasco WA, Phan SH, Krutzsch H, Showell HJ, Feltner DE, Nairn R, Becker EL, Ward PA: Purification and identification of formyl-methionyl-leucyl-phenylalanine (fMet-Leu-Phe) as the major peptide neutrophil chemotactic factor produced by *Escherichia coli*. J Biol Chem 1984, 259:5430–5439
25. Redl H, Flynn PJ, Lamche H, Scheisser A, Schlag G, Hammerschmidt DE: Aggregation, chemotaxis, and chemiluminescence of canine granulocytes. Studies utilizing improved cell preparation techniques. Inflammation 1983, 7:67–80
26. Gray GD, Knight KA, Nelson RD, Herron MJ: Chemotactic requirements of bovine leukocytes. Am J Vet Res 1982, 43:957–759
27. Chenoweth DE, Lane TA, Rowe JG, Hugh TE: Quantitative comparisons of neutrophil chemotaxis in four animal species. Clin Immunol Immunopathol 1980, 15:525–535
28. Gray GD, Ohlmann GM, Morton DR, Schaub RG: Feline polymorphonuclear leukocytes respond chemo-

tactically to leukotriene B4 and activated serum but not to fMet-Leu-Phe. Agents Actions 1986, 18:401–406
29. Snyderman R, Pike MC: N-formylmethionyl peptide receptors on equine leukocytes initiate secretion but not chemotaxis. Science 1980, 209:493–495
30. Becker EL: Chemotactic factors of inflammation. Trends Pharmacol 1983, 4:223–225
31. Gnessi L, Ruff MR, Fraioli F, Pert CB: Demonstration of receptor-mediated chemotaxis by human spermatozoa: A novel quantitative bioassay. Exp Cell Res 1985, 161:219–230
32. Iqbal M, Shivaj S, Vijayasarathy S, Balaram P: Synthetic peptides as chemoattractants for bull spermatozoa: Structure-activity correlations. Biochem Biophys Res Commun 1980, 96:235–242
33. Marasco WA, Fantone SC, Ward PA: Spasmogenic activity of chemotactic N-formylated oligopeptides: Identity of structure-function relationships for chemotactic and spasmogenic activities. Proc Natl Acad Sci USA 1983, 79:7470–7473
34. Burka JF: Effects of compound 48/80 and formyl-methionyl-leucyl-phenylalanine on isolated guinea pig airways. Int Arch Allergy Appl Immunol 1984, 73:309–313
35. Hamel R, Ford-Hutchinson AW, Lord A, Cirino M: Bronchoconstiction induced by N-formyl-methionyl-leucyl-phenylalanine in the guinea pig: involvement of arachidonic acid metabolites. Prostaglandins 1984, 28:43–56
36. Boukill MA, Bureau M, Lagenta V, Lefort J, Lellouch-Tubiana A, Malan-Chere E, Vargaftig BB: Pharmacological modulation of the effects of N-formyl-L methionyl-L-leucyl-L-phenylalanine in guinea pigs: Involvement of the arachidonic acid cascade. Br J Pharmacol 1986, 89:349–359
37. Sha'afi RI, Molski TFP: Activation of the neutrophil: Surface reactions of immunologically relevant cells. Prog Allergy (In press)
38. Stryer L, Bourne HR: G proteins: a family of signal transducers. Ann Rev Cell Biol 1986, 2:391–419
39. Koo C, Lefkowitz RJ, Snyderman R: Guanine nucleotides modulate the binding affinity of the oligopeptide chemoattractant receptor on human polymorphonuclear leukocytes. J Clin investig 1983, 72:748–753
40. Hyslop PA, Oades ZG, Jesaitis AJ, Painter RG, Cochrane CG, Sklar LA: Evidence for N-formyl chemotactic peptide-stimulated GTPase activity in human neutrophil homogenates. FEBS Lett 1984, 166:165–169
41. Okajima F, Ui M: ADP-ribosylation of the specific membrane protein by islet-activating protein, pertussis toxin, associated with inhibition of a chemotactic peptide-induced arachidonate release in neutrophils: A possible role of the toxin substrate in Ca^{2+}-mobilizing biosignaling. J Biol Chem 1984, 59:13863–13871
42. Feltner, DE, Smith RH, Marasco WA: Characterization of the plasma membrane bound GTPase from rabbit neutrophils: 1. Evidence for an N_i-like protein coupled to the formyl peptide, C5a, and leukotriene B4 chemotaxis receptors. J Immunol 1986, 137:1961–1970
43. Pelz C, Matsumoto T, Sha'afi RI: Characterization of the membrane associated GTPase activity in rabbit neutrophils (Abstr). Fed Proc 1987, 46:1037
44. Becker EL, Kermode JC, Naccache PH, Yassin R, Marsh ML, Munoz JJ, Sha'afi RI: Inhibition of neutrophil granule enzyme secretion and chemotaxis by pertussis toxin. J Cell Biol 1985, 100:1641–1646
45. Naccache PH, Molski MM, Volpi M, Becker EL, Sha'afi RI: Unique inhibitory profile of platelet activating factor induced calcium, mobilization, polyphosphoinositide turnover and granule enzyme secretion in rabbit neutrophils toward pertussis toxin and phorbol ester. Biochem Biophys Res Commun 1985, 26:482–492
46. Becker EL: Leukocyte stimulation: Receptor, membrane and metabolic events. Fed Proc 1986, 45:2148–2150
47. Spangrude GJ, Sacchi F, Hill HR, Van Epps DE, Daynes RA: Inhibition of lymphocyte and neutrophil chemotaxis by pertussis toxin. J Immunol 1985, 135:4135–4143
48. Lad PM, Olson CV, Grewal IS: A step sensitive to pertussis toxin and phorbol ester in human neutrophils regulates chemotaxis and capping but not phagocytosis. FEBS Lett 1986, 200:91–96
49. Kanaho Y, Huang CK, Becker EL: Biochemical discrimination between guanine nucleotide-binding protein of rabbit neutrophils and Gi, Go and transducin (Abstr). Fed Proc 1986, 45:1523
50. Barrowman MM, Cockcroft S, Gomperts: Two roles for guanine nucleotides in the stimulus-secretion sequence of neutrophils. Nature 1986, 319:504–507
51. Verghese M, Ushing RJ, Snyderman R: A pertussis toxin/choleratoxin-sensitive N protein may mediate chemoattractant receptor signal transducin. Biochem Biophys Res Commun 1986, 138:887–894
52. Gierschik P, Fallon J, Milligan G, Pines M, Gallin JI, Spiegel A: Immunochemical evidence for a novel pertussis toxin substrate in human neutrophils. J Biol Chem 1986, 261:8058–8062
53. Fallon J, Malich A, Milligan G, Unson C, Kahn R, Goldsmith P, Spiegel A: Detection of the major pertussis toxin substrate of human leukocytes with antisera raised against synthetic peptides. FEBS Lett 1986, 209:352–356

Acknowledgments

I wish to take this opportunity to thank my collaborators. In many ways, they have given me more than I have given them.