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ANTI-IDIOTYPE AS ANTIBODY AGAINST THE FORMYL PEPTIDE CHEMOTAXIS RECEPTOR OF THE NEUTROPHIL[1]

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Anti-idiotypic antibodies have been produced in mice, guinea pigs, and goat against rabbit antibodies to the chemoattractant f Met-Leu-Phe. The anti-idiotypic antibodies were demonstrated by their capacity in the presence of preimmune rabbit serum to block the binding of ^{125}I anti-f Met-Leu-Phe to f Met-Leu-Phe-human transferrin absorbed to microtiter plates. Goat anti-idiotypic antibodies were further demonstrated by their ability, when absorbed to *Staphylococcus aureus*, to selectively bind ^{125}I anti-f Met-Leu-Phe.

In addition, some of the goat anti-idiotypic IgG was able to bind nearly all rabbit and rat anti-f Met-Leu-Phe antibodies examined. This was shown by 1) the ability of antibodies produced in rabbits immunized with either f Met-Leu-Phe conjugated to goat IgG or f Met-Leu-Phe conjugated to keyhole limpet hemocyanin (KLH) when adsorbed to *S. aureus* to bind the ^{125}I F(ab')$_2$ goat anti-idiotype, and 2) the ability of various rat anti-f Met-Leu-Phe antibodies to block the binding of ^{125}I rabbit anti-f Met-Leu-Phe to goat anti-idiotype absorbed to *S. aureus*.

Finally, goat anti-idiotypic antibodies can also cross-react with the receptor on the rabbit neutrophil for the formyl peptides as evidenced by 1) the direct binding of goat anti-idiotypic IgG to the PMN, 2) the ability of F(ab')$_2$ fragments to goat anti-idiotype to partially inhibit the binding of f Met-Leu-(^{3}H)Phe to rabbit PMN, and 3) loss of anti-idiotype and anti-PMN receptor activity after passage over an anti-f Met-Leu-Phe column.

These data support Jerne's hypothesis of "internal image" and suggest a feasible experimental approach for producing anti-cell receptor antibody without purifying the receptor.

In the preceding paper, we showed that antibody (idiotype) against the chemotactic formyl peptide, formylmethionyl-leucyl-phenylalanine (f Met-Leu-Phe)[3] is extremely similar in binding specificity to the neutrophil surface receptor for chemotactic formyl peptides (1). The similar specificities suggest that certain ligand recognition structures or conformations are conserved in both receptor sites. In this communication, we report on the production of anti-idiotypic antibodies raised against affinity column purified rabbit anti-f Met-Leu-Phe (idiotype). We show that one goat produced anti-idiotypic antibodies that were able to bind nearly all rabbit and rat anti-f Met-Leu-Phe antibodies tested. In addition, these anti-idiotypic antibodies were able to bind to the rabbit neutrophil formyl peptide receptor. Whether these results demonstrate the conserved idiotypic nature of these formyl peptide binding sites or the existence of a subpopulation of "internal image" anti-idiotypic antibodies will be discussed.

MATERIALS AND METHODS

Preparation of immunogen. The antibody used as immunogen was the fifth bleeding from a single rabbit immunized with fMLP$_{10}$-BSA[3] and has been extensively characterized (1). For purification, the rabbit anti-f Met-Leu-Phe antiserum was precipitated with a final concentration of 50% saturated ammonium sulfate, the precipitate dissolved in 0.01 M phosphate buffer, dialyzed, and applied to a DEAE-cellulose (Whatman-DE52, Kent, England) column. The "break-through" peak was concentrated in an Amicon concentrator, Diaflo PM10 membrane (Amicon Corporation, Lexington, MA) followed by gel filtration using G-150 or Sephacryl S-200 (Pharmacia, Uppsala, Sweden). The IgG obtained showed only one precipitin line with IgG mobility when analyzed in immunoelectrophoresis with goat anti-rabbit whole serum and goat anti-rabbit IgG (Cappell Laboratories, Cochranville, PA) and showed only IgG on 7.5 or 10% sodium dodecyl sulfate (SDS) slab gel electrophoresis (2). The antibody activity was routinely determined during purification by titration of the purified IgG with 0.88 pmol f Met-Leu-(^{3}H)Phe (1).

Affinity column purification of anti-f Met-Leu-Phe. The purified IgG was filtered over a column of f Met-Leu-Phe-human transferrin Sepharose 4B previously equilibrated in borate-saline buffer (160 mM H_3BO_3, 140 mM NaCl, 22 mM NaOH, pH 8.0). This removed over 95% of f Met-Leu-Phe binding activity. The anti-f Met-Leu-Phe was eluted with 0.1 M glycine HCl, pH 2.6, immediately neutralized with 1 M NaOH, followed by dialysis and gel filtration in borate-saline buffer. Recovery of specific activity ranged from 20 to 40%. Elution with 5 M guanidine HCl (pH unadjusted) gave greater recovery of activity, but more protein denaturation occurred. All attempts to recover specific antibody from AH-Sepharose 4-B (Pharmacia, Uppsala, Sweden) to which f Met-Leu-Phe had been directly attached by the carbodiimide procedure (3) failed using glycine HCl (pH 2.6), 8 M urea, or (10^{-4} M) f Met-Leu-Phe.

Preparation of immunoabsorbants. For the preparation of f Met-Leu-Phe immunoabsorbant columns, f Met-Leu-Phe was first covalently bound to either goat IgG (IgG-fMLP) or human transferrin (transferrin-fMLP) as previously described. The f Met-Leu-Phe conjugate was coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) (3) at a final protein concentration of 2 mg/ml gel. Excess protein was washed away with $NaHCO_3$ buffer, and the remaining active groups were blocked by addition of 0.2 M glycine (pH 8.2). The Sepharose-antigen conjugate was stored in borate-saline buffer until ready for use. The identical procedure was used for the preparation of columns of rabbit immunoglobulin (IgG, IgM, and IgA), rabbit anti-f Met-Leu-Phe or rabbit whole serum.

Immunization. A single goat was immunized with the affinity column-purified rabbit anti-f Met-Leu-Phe (500 μg) in a 1:1 mixture with complete Freund's adjuvant (Difco Company, Detroit, MI) by multiple intradermal injections. This was repeated two times at 2-wk intervals except that incomplete Freund's adjuvant was employed. The goat was then bled at weekly intervals for 4 wk. Four months after the primary immunization, a booster immunization was given with 2 mg anti-f Met-Leu-Phe in complete Freund's adjuvant, and the goat was bled weekly for 4 wk.

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[3] Abbreviations used in this paper: f Met-Leu-Phe, fMLP, formylmethionyl-leucyl-phenylalanine; EDTA, ethylene diaminetetraacetic acid; KLH, keyhole limpet hemocyanin; PMN, polymorphonuclear leukocytes; fMLP$_x$-carrier protein conjugate, where X refers to the mean number of fMLP molecules bound per molecule of carrier protein.

For the production of anti-idiotype antibody in mice, the protocol described by Tung *et al.* (4) was used. In brief, four CDI mice were immunized i.p. at weekly intervals for 5 wk with 250 μg protein in a 1:9 mixture with complete Freund's adjuvant in a total volume of 0.2 ml. Appreciable ascites developed in all mice after 6 wk, and the fluids were subsequently pooled.

For the production of anti-idiotype antibody in guinea pigs, four Hartley strain guinea pigs were immunized i.p. with 250 μg protein in 1:9 mixture with complete Freund's adjuvant (total volume 0.6 ml) for 4 wk (5, 6). After an additional 4-wk rest period, an identical injection was given, and the guinea pigs were tapped 1 wk later. By using this protocol, large volumes of ascites fluid could be obtained (i.e., 80 to 100 ml/tap).

Purification of goat anti-idiotypic IgG. Goat antiserum directed against rabbit anti-f Met-Leu-Phe was rendered idiotypic specific by passage over a rabbit immunoglobulin (IgG, IgM, and IgA) column to remove antibodies to the common determinants on rabbit IgG, e.g. constant region determinants. This antiserum was further absorbed over an f Met-Leu-Phe-human transferrin column to eliminate possible antibodies that might react directly with this protein conjugate. After absorption, no precipitin line could be detected on Ouchterlony gels run against rabbit whole serum, rabbit IgG, or human transferrin-f Met-Leu-Phe.

Goat IgG was purified by precipitation with 35% saturated ammonium sulfate (7), followed by chromatography over DEAE-cellulose and gel filtration on Sephacryl S-200. Ouchterlony tests using rabbit anti-goat whole serum and rabbit anti-goat IgG gave a single line of identity against the purified immunoglobulin preparation.

$F(ab')_2$ fragments of goat IgG were produced by a method described by Nisonoff *et al.* (8) using porcine pepsin (Worthington Biochemical Corporation, Freeport, NJ; 2800 U/mg). After dialysis against borate-saline buffer, the $F(ab')_2$ was purified over an S-200 column. Fractions were subsequently analyzed for purity using 7.5% SDS gels, as described (2).

For the production of monovalent F(ab') fragments, the $F(ab')_2$ was dialyzed against 0.1 M phosphate buffer, pH 6.5, containing 0.1 mM EDTA[3] (9). Cysteine reduction was performed at 37°C by addition of cysteine (pH 6.5) to a final protein-to-cysteine ratio of 1:100 (w/w). The reaction was stopped at various times by addition of 2-iodoacetamide in a 50-fold molar excess of the cysteine. Analysis on 10% SDS polyacrylamide gels confirmed the complete reduction and alkylation (10).

Preparation of immunoglobulin-bearing staphylococcus aureus. Staphylococcus aureus (Cowan I) bearing protein A was obtained from the clinical microbiology department of this university. The bacteria were grown at 37°C in beef heart culture broth (Difco Laboratories, Detroit, MI) for 24 hr and harvested by centrifugation at 12,000 × G for 30 min. Bacteria were resuspended and washed three times in phosphate-buffered saline (PBS; 5 mM PO_4, 0.145 M NaCl, pH 7.2) supplemented with 2% sodium azide. The bacteria were then fixed with 5% glutaraldehyde in PBS, sonicated, and resuspended in PBS plus 2% NaN_3. Cell density was adjusted to 1×10^{10} bacteria/ml by assuming the optical density at 600 nm for 1×10^8 cells/ml = 0.3. Before use, bacteria were washed and resuspended in 10 mM phosphate buffer containing 0.1 M NaCl, 1 mM EDTA, and 0.1% Triton X-100, pH 7.5. Goat or rabbit antiserum or preimmune serum (0.5 ml) was added to 0.5 ml of bacteria suspension and incubated at 25°C for 30 min. The mixtures were diluted with 3.5 ml buffer and centrifuged as above, and the supernatants were aspirated. The bacteria were washed twice by resuspension and centrifugation, and then resuspended and stored at 4°C in 0.5 ml buffer.

Radiolabeling of proteins. Affinity column-purified anti-f Met-Leu-Phe, goat anti-rabbit IgG, preimmune goat $F(ab')_2$, goat anti-idiotypic $F(ab')_2$, and protein A were iodinated using an Enzymobead Radioiodination Reagent Kit (Bio-Rad Laboratories, Richmond, CA). The proteins were iodinated to a specific activity of 1000 to 3000 cpm/ng as determined by precipitating the protein at 4°C with 10% final concentration of trichloroacetic acid (TCA). Iodinated protein A (70 to 100 μCi/μg) was obtained from New England Nuclear (Boston, MA), as was f Met-Leu-(^{3}H)Phe (51.6 mCi/mmol).

Indirect anti-idiotype assay. For the analysis of anti-idiotype activity, both indirect and direct assay systems were utilized. The indirect assay was performed on disposable polyvinylchloride-coated microtiter plates (Dynateck Laboratories, Alexandria, VA) in a modified procedure of Klinman *et al.* (11). In brief, a solution of 3 mg/ml transferrin-fMLP in borate-saline buffer was incubated overnight at 25°C in the wells of a microtiter plate. The wells were washed four times with borate-saline buffer, filled with 2% horse serum in borate-saline buffer to saturate the hydrophobic protein binding sites, incubated at 25°C overnight, then washed four times with borate-saline buffer. When not immediately used, the plates were filled with 2% horse serum and placed at 4°C to minimize protein desorption from the plate. The assay was preformed by incubating in the microtiter wells containing fMLP-transferrin, 125 ng rabbit ^{125}I anti-f Met-Leu-Phe in 50 μl buffer with 50 μl of six serial four-fold dilutions of the anti-idiotypic serum or ascites fluid or buffer. All experiments were done in the presence of 5 μl preimmune rabbit serum (1000-fold IgG excess) to insure that subgroup and nonidiotypic determinants on the IgG were not being recognized. A standard curve was constructed by determining the amount of label bound at varying concentrations of labeled protein added. After 3 hr incubation at 25°C, the wells were emptied and washed four times with borate-saline buffer followed by eight times with distilled H_2O. The wells were then cut out and counted in a gamma counter (Searle Analytic, Inc., Chicago, IL, model 1195). The dilution of anti-idiotypic serum that gave 50% inhibition (ID_{50}) of ^{125}I-anti-f Met-Leu-Phe binding to the absorbed f Met-Leu-Phe-transferrin was used as the index of inhibitory activity.

Direct anti-idiotype assay. For the direct anti-idiotype assay, 50 μl of varying dilutions of the suspension (10^{10} cells/ml) of anti-idiotype loaded *S. aureus* in 1.5 ml polypropylene microcentrifuge tubes were incubated at 4°C overnight with 50 μl containing 125 ng rabbit ^{125}I-anti-f Met-Leu-Phe (12). Preliminary experiments determined that this procedure would minimize dissociation of bound anti-idiotype. In separate experiments, the ^{125}I anti-f Met-Leu-Phe was shown not to directly bind to the *S. aureus* under these circumstances. Acid elution of immunoglobulins, as used to prepare the anti-f Met-Leu-Phe, has been shown to disrupt Fc function (13) and significantly decrease protein A-binding capacity (unpublished observations). Fifty microliters of the supernatant fluid were removed and counted in a gamma counter after centrifugation for 3 min in a Brinkman centrifuge (model 3200, Brinkman Instruments, Westbury, NY). Preimmune IgG-loaded *S. aureus* served as control throughout these experiments.

Binding of ^{125}I $F(ab')_2$ goat anti-idiotype to various rabbit anti-f Met-Leu-Phe antibodies. Various rabbit anti-f Met-Leu-Phe antisera or preimmune serum (0.5 ml) were added to 0.5 ml of a 10^{10}/ml *S. aureus*/suspension. The immunoglobulin-loaded bacterial suspensions were prepared identically to that for the goat antiserum.

Fifty microliters containing 640 ng ^{125}I-$F(ab')_2$ goat anti-idiotypic IgG was added to 50 μl of various dilutions of the rabbit immunoglobulin-loaded *S. aureus* suspensions, and the assay was performed as described for the direct anti-idiotypic assay. For analysis of the anti-f Met-Leu-Phe antiserum raised against $fMLP_{12}$-goat IgG, 50 μl of a 100-fold excess of preimmune goat $F(ab')_2$ were added to insure idiotypic binding specificity.

Inhibition by rat anti-f Met-Leu-Phe antibodies of ^{125}I anti-f Met-Leu-Phe binding to goat anti-idiotype. One milliliter of goat anti-idiotypic antiserum was incubated with an equal volume of 10^{10} bacteria at 37°C for 30 min and treated as described. The washed bacteria were resuspended to 10^{10} cells/ml. Various dilutions of 50 μl of different rat anti-f Met-Leu-Phe antisera or rat preimmune serum were incubated overnight at 4°C with 125 ng rabbit ^{125}I-anti-f Met-Leu-Phe and 50 μl of bacterial suspension. The microfuge tubes were then centrifuged, and a 25-μl aliquot of supernatant was removed and counted.

Neutrophil binding. Rabbit peritoneal neutrophils, obtained as described previously (14), were suspended in Hanks' buffer supplemented with 1.6 mM Ca^{++} at a cell concentration of 5×10^7 cells/ml. For the standard binding assay (15), 5×10^6 cells (in a 12 x 75 mm glass tube) and 0.88 pmol f Met-Leu-Phe-(^{3}H)-Phe (specific activity 56.9 ci/mmol; New England Nuclear, Boston, MA) were incubated at 4°C in 105 μl Hanks' buffer plus Ca^{++}. At the end of 30 min, 2 ml Hanks' buffer were added, and the suspension was agitated on a Vortex mixer and rapidly filtered through a Whatman GF/C filter. The filter was washed with 8 ml in 2-ml aliquots of ice-cold Hanks' buffer, then added to a liquid scintillation vial containing 5 ml of Aquasol (New England Nuclear) and counted. Nonspecific binding, defined as the cpm bound in the presence of 1000-fold excess unlabeled peptide, was always less than 10% of the total binding. Specific binding only is reported in this paper and is defined as the total cpm of f Met-Leu-(^{3}H)Phe bound minus the cpm due to nonspecific binding.

For the binding studies involving immunoglobulin, the cells were pretreated with immunoglobulin (preimmune or anti-idiotype) or unlabeled f Met-Leu-Phe at 4°C for 30 min before the addition of f Met-Leu(^{3}H)Phe, and the mixture was incubated for 20 min. The cells were then washed and filtered as described.

Detection of goat anti-idiotype binding to PMN by ^{125}I-protein A. ^{125}I-Protein A was bound to neutrophils by a modification of the procedure of McCallister *et al.* (16). In brief, $1.5 \times ^7$ ml washed rabbit neutrophils in 200 μl Hanks' buffer containing 1 mM NaN_3 were incubated at 4°C for 30 min with an equal volume of varying dilutions of preimmune or anti-idiotypic goat IgG. The cell suspension was centrifuged at 3000 rpm for 5 min at 4°C, washed twice, and resuspended in 200 μl of cold Hanks' buffer containing 1 μg of ^{125}I-protein A (specific activity 5×10^6 cpm/μg protein). The suspension was incubated for 30 min at 4°C and washed two times. The final pellet was transferred to a fresh tube and the ^{125}I-radioactivity was counted. Goat IgG1 and IgG2 are both known to bind protein A (17), and SDS slab gel electrophoresis did not reveal significant alterations in the IgG subclass components IgG1 and IgG2 during the immune response. Thus, the latter could not complicate the analysis of ^{125}I-protein A binding.

RESULTS

Inhibition of ^{125}I anti-f Met-Leu-Phe binding to f Met-Leu-Phe-transferrin by various anti-idiotypic antibodies. Figure 1 demonstrates the ability of various anti-idiotypic antibodies to inhibit the binding of ^{125}I anti-f Met-Leu-Phe to f Met-Leu-Phe-human transferrin absorbed to a microtiter plate. As can be seen, goat,

guinea pig, and mouse anti-idiotypic antibodies are inhibitory. The slopes of the inhibition curves are relatively constant, even though the titer of the various anti-idiotypic preparation vary significantly. Goat anti-idiotype showed the highest titer of inhibitory activity and was therefore used in all subsequent studies. The concentration of goat anti-idiotypic activity measured in this assay (11, 18) ranged from 5 μ/ml for earlier bleeds to 150 μg/ml for bleeds after the secondary booster immunization.

The specificity of the assay system was demonstrated in a number of ways. In addition to the various anti-idiotypic antibodies, free f Met-Leu-Phe and various f Met-Leu-Phe conjugates blocked ^{125}I-anti-f Met-Leu-Phe binding, as did nonradiolabeled rabbit anti-f Met-Leu-Phe. However, the rabbit preimmune or nonimmune IgG did not compete for binding. In addition, plates absorbed with human tranferrin alone or 2% horse serum alone did not bind the ^{125}I anti-f Met-Leu-Phe.

Direct binding of ^{125}I anti-f Met-Leu-Phe to anti-idiotype loaded S. Aureus. Figure 2 shows the direct binding of ^{125}I anti-f Met-Leu-Phe to goat anti-idiotype-loaded *S. aureus*. Preimmune goat IgG-loaded *S. aureus* did not bind ^{125}I anti-f Met-Leu-Phe. This assay allowed the binding capacity of the anti-idiotypic antiserum for idiotype to be calculated directly. The results are in good agreement with the inhibition assay. For early bleeds, 1 ml of anti-idiotype bound 10 μg idiotype, and for bleeds after the secondary boost, 125 to 150 μg idiotype were bound.

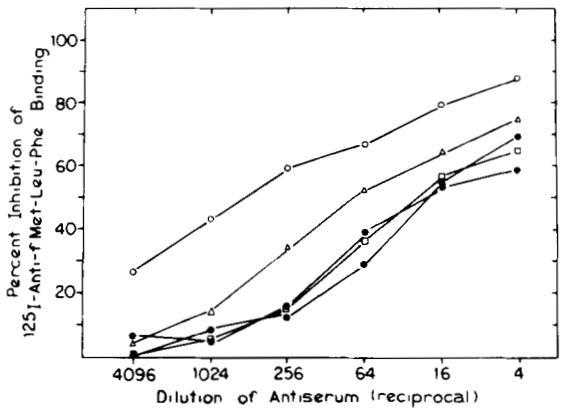

Figure 1. Ability of anti-idiotypic antibodies from various species (○) goat; (△) guinea pig 1; (•) guinea pig 2; (□) guinea pig 3; (●) pool from four CDL mice, to inhibit binding of ^{125}I anti-f Met-Leu-Phe to f Met-Leu-Phe transferrin.

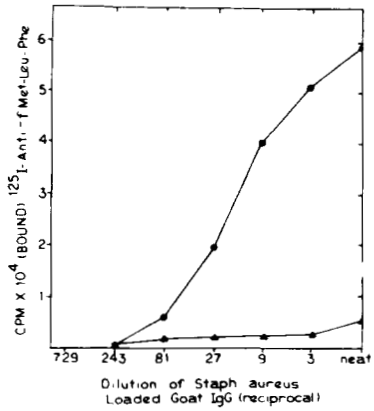

Figure 2. Ability of goat IgG anti-idiotype (●) and inability of goat preimmune IgG (▲) to directly bind ^{125}I rabbit anti-f Met-Leu-Phe. Both goat IgG anti-idiotype and preimmune IgG were absorbed to *S. aureus*.

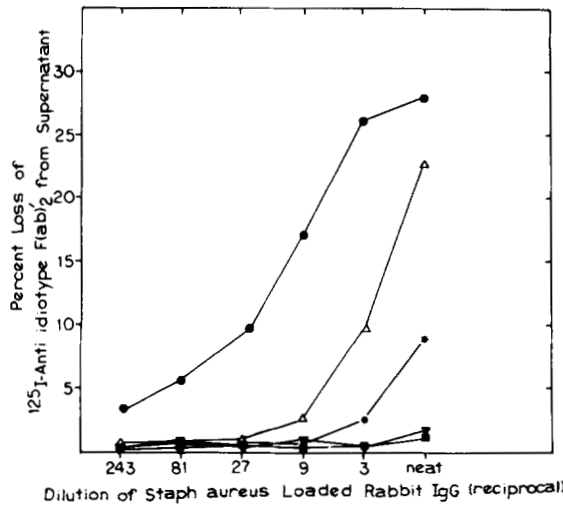

Figure 3. Ability of ^{125}I F(ab'$_2$) goat anti-idiotype to bind to IgG anti-f Met-Leu-Phe produced in three different rabbits immunized with f Met-Leu-Phe conjugated to different carrier proteins: (●——●) anti-fMLP$_{10}$-BSA; (△——△) anti-fMLP$_{147}$-KLH; (•——•) anti fMLP$_{12}$-goat IgG; (■——■) anti fMLP$_5$-rabbit albumin; (▼——▼) preimmune rabbit IgG. The IgG from each antiserum was adsorbed to *S. aureus*.

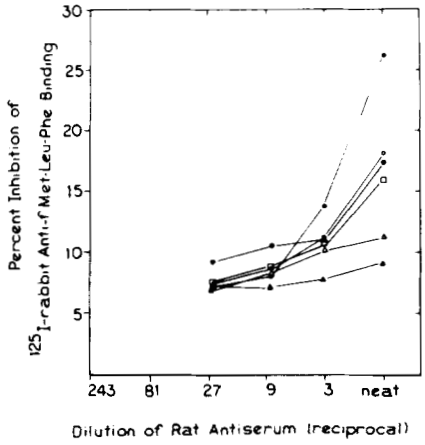

Figure 4. The ability of various rat anti-f Met-Leu-Phe antisera to inhibit the binding of ^{125}I rabbit anti-f Met-Leu-Phe to goat anti-idiotype raised against rabbit anti-f Met-Leu-Phe. The sera are from rat, (○) R 1: (●) R 2; (•) R 3; (△) R 4; (□) R 5; and (▲) pool of preimmune rat serum.

Binding of ^{125}I F(ab')$_2$ goat anti-idiotype to various rabbit anti-f Met-Leu-Phe antibodies. We investigated the possibility that goat anti-idiotype raised against anti-f Met-Leu-Phe produced in a single rabbit could also bind to anti-f Met-Leu-Phe antibodies produced in different rabbits. Figure 3 demonstrates the ability of ^{125}I F(ab')$_2$ goat anti-idiotype to bind to anti-f Met-Leu-Phe produced in three different rabbits immunized with f Met-Leu-Phe conjugated to three different carrier proteins. The degree of ^{125}I F(ab')$_2$ anti-idiotype binding to anti-f Met-Leu-Phe directly reflects the anti-f Met-Leu-Phe titers of the rabbit antisera previously reported (10). The inability to detect binding to anti-f Met-Leu-Phe raised against fMLP$_5$-rabbit albumin is probably due to the low titer of the anti-f Met-Leu-Phe in this antiserum. Thus, rabbit anti-f Met-Leu-Phe antibodies with similar peptide binding reactivity are able to bind at least a portion of the goat anti-idiotypic IgG.

Inhibition by rat anti-f Met-Leu-Phe antibodies of ^{125}I anti-f Met-Leu-Phe binding to goat anti-idiotype. Antiserum from five DAL rats immunized with FMLP$_{10}$BSA were analyzed, as previously described (1) for their f Met-Leu(^{3}H)Phe-binding capacity. All but one rat (R$_4$) produced anti-f Met-Leu-Phe. We next

investigated the ability of the various rat anti-f Met-Leu-Phe antiserums to block the binding of rabbit ^{125}I-anti-f Met-Leu-Phe to goat anti-idiotype absorbed to *S. aureus*. As seen in Figure 4, all rat antisera containing anti-f Met-Leu-Phe binding activity inhibited the binding of the rabbit ^{125}I anti-f Met-Leu-Phe to goat anti-idiotype. In addition, only a small percentage of binding inhibition is observed.

Furthermore, the ability to block the idiotype/anti-idiotype binding was again directly proportional to the anti-f Met-Leu-Phe titer (10). Thus, these data are similar to our findings with the rabbit antibodies and afford additional evidence of the ability of various anti-f Met-Leu-Phe combining sites to interact with some of the goat anti-idiotypic IgG.

Inhibition of f Met-Leu-Phe-(^{3}H)Phe binding to rabbit neutrophil by F(ab')$_2$ goat anti-idiotype. As described in the preceding paper, the specificity of the anti-f Met-Leu-Phe binding site strongly resembles that of the rabbit neutrophil formylpeptide receptor in the proposed first four critical areas for peptide-receptor interaction (1). Therefore, we next investigated the possibility that the anti-idiotypic antibodies could also cross-react with the neutrophil formylpeptide receptor.

Figure 5 shows that F(ab')$_2$ fragments of goat anti-idiotype can compete, albeit only partially, with f Met-Leu-(^{3}H)Phe for binding to the rabbit neutrophil. Preimmune F(ab')$_2$ fragments had no effect. In four identical experiments (data not shown), anti-idiotypic F(ab'), F(ab')$_2$, or IgG competed for between 25 and 35% of the total available binding sites. We have not seen complete inhibition of f Met-Leu-(^{3}H)Phe binding to polymorphonuclear leukocytes (PMN) by anti-idiotype even at the highest attainable protein concentrations. The concentration range required for inhibition by anti-idiotype of f Met-Leu-(^{3}H)Phe binding to PMN is similar to the range where anti-idiotype induced inhibition of ^{125}I anti-f Met-Leu-Phe binding to fMLP-human transferrin occurs (data not shown).

Direct binding of anti-idiotype to the neutrophil, detected by ^{125}I-protein A. Figure 6 demonstrates the ability of IgG anti-idiotype antibodies produced in the goat to bind to rabbit neutrophils. As can be seen, preimmune goat IgG can also bind to the neutrophil in a dose-dependent fashion but to a lesser extent than the anti-idiotypic IgG at each protein concentration tested. No attempt to destroy the Fc receptors on the neutrophil was made; thus, the binding of the preimmune IgG was possible via the Fc receptor, even though goat IgG has been reported not to bind to the Fc receptor on the rabbit lymphocyte (19). Baseline values for ^{125}I-protein A binding to neutrophils not incubated with goat immunoglobulin varied considerably; however, this ^{125}I-protein A binding was displaceable by even low concentrations of goat IgG (i.e., 1 μg/ml). Large differences between preimmune and anti-idiotype IgG binding to PMN are only seen at high protein concentrations, reflecting the low concentration and possible low affinity of this antibody for the formyl peptide receptor.

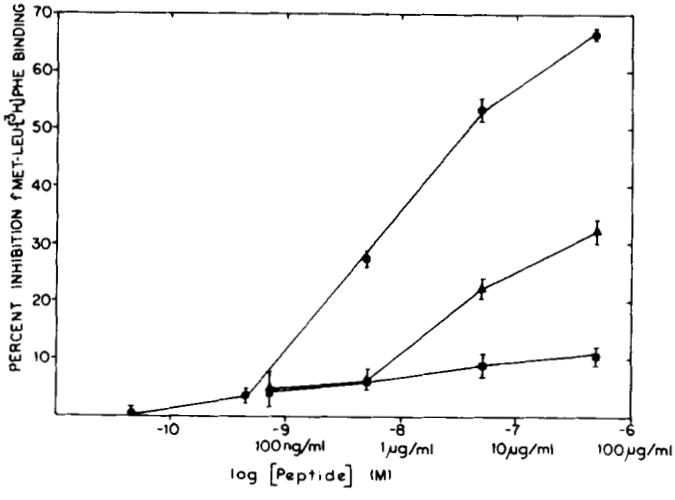

Figure 5. Inhibition of f Met-Leu-(^{3}H)Phe binding to PMN by f Met-Leu-Phe (●——●) and F(ab')$_2$ fragments of goat anti-idiotypic antibodies against anti-f Met-Leu-Phe (▲——▲) and the lack of effect of pre-immune F(ab')$_2$ (■——■). Error bars ± standard deviation.

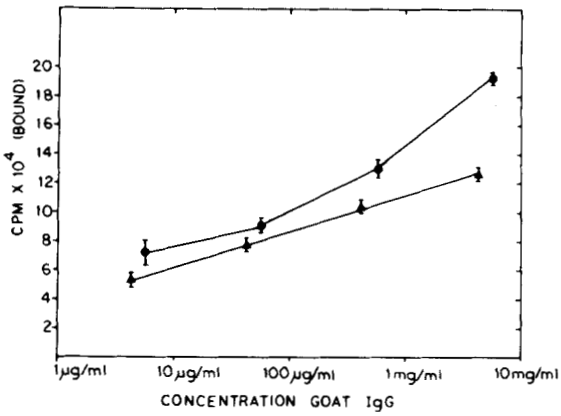

Figure 6. The binding of goat anti-idiotype IgG fractions as detected by ^{125}I-protein A. Preimmune (▲——▲) and anti-idiotypic (●——●) fractions were separately incubated with rabbit neutrophils and the amount of IgG bound to the cells was measured by the addition of ^{125}I-protein A. Error bars ± standard deviation.

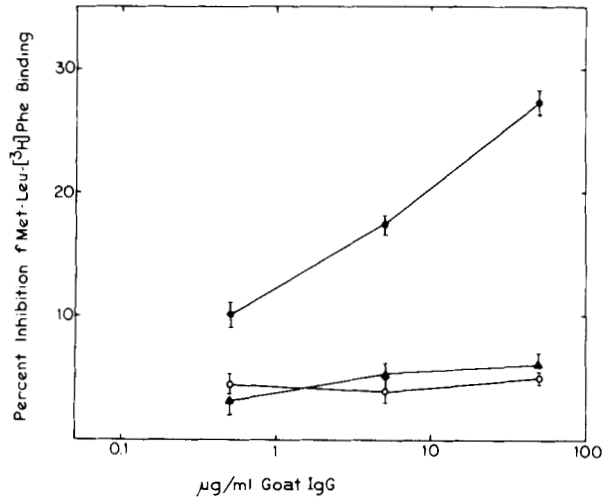

Figure 7. Depletion of anti-formyl peptide receptor activity after passage through a rabbit anti-f Met-Leu-Phe (idiotype) affinity column. (●——●) F(ab')$_2$ goat anti-idiotype before passage; (▲——▲), F(ab')$_2$ goat anti-idiotype after passage; (○——○). F(ab')$_2$ goat pre-immune. Error bars ± standard deviation.

An estimate of the number of binding sites per cell bound by anti-idiotype was made based on the differences in binding of preimmune and anti-idiotype to the PMN. These values (7000 to 65,000 sites/cell) are well within the range we and others have found for the number of formyl peptide receptors (15, 20).

Depletion of anti-idiotype and anti-formyl peptide receptor activity by passage through an anti-f Met-Leu-Phe (idiotype) affinity column. Figure 7 shows the results of passage of anti-idiotype through an anti-f Met-Leu-Phe column. As can be seen, complete loss of anti-formyl peptide receptor activity occurred thus demonstrating that the anti-PMN receptor component of the anti-idiotype IgG could also bind the anti-f Met-Leu-Phe antibody. Furthermore, this same material was also

depleted 200-fold in its ability to block ^{125}I anti-f Met-Leu-Phe binding to f Met-Leu-Phe human transferrin compared with the active material recovered from the affinity column by acid elution. The latter was enriched 12-fold over unabsorbed material in the indirect anti-idiotype assay just described, and more than fivefold enriched in its ability to block f Met-Leu-(^{3}H)Phe binding to the PMN (data not shown).

DISCUSSION

The results we have just described illustrate the production of anti-idiotypic antibodies to rabbit anti-f Met-Leu-Phe in mice, guinea pigs, and a goat by using different immunization schemes. In the goat, the anti-idiotypic IgG titer, which increased during the primary immunization, sharply rose more than 10-fold after the secondary boost with antigen.

Two different systems were used to demonstrate the activity of the anti-idiotypic antibody. In the first, the appropriately absorbed goat anti-idiotypic IgG blocked the binding of ^{125}I anti-f Met-Leu-Phe to $FMLP_5$-human transferrin absorbed on a microtiter plate. The preliminary absorption and the presence of 5% preimmune rabbit serum ensured idiotypic specificity. In the second, the goat anti-idiotype absorbed on to *S. aureus* bound rabbit ^{125}I anti-f Met-Leu-Phe IgG but not ^{125}I-preimmune rabbit IgG, again showing idiotypic specificity. Furthermore, in both systems a high (10^{-4} M) final concentration of f Met-Leu-Phe inhibited greater than 90% of the binding (data not shown), indicating that the vast majority of the anti-idiotypic antibody is directed to or near the anti-f Met-Leu-Phe combining site.

The goat anti-idiotypic antibodies raised against anti-$fMLP_{10}$-BSA are also able to bind the anti f Met-Leu-Phe antibodies produced in different rabbits even when the latter were immunized with f Met-Leu-Phe coupled to different carrier proteins, e.g., $fMLP_{12}$ goat IgG and $fMLP_{147}$-KLH.[3] Moreover, a small proportion of the goat anti-idiotypic IgG also interacts with the rat anti-f Met-Leu-Phe antibodies.

In addition to reacting with anti-f Met-Leu-Phe intra- and inter-species idiotypes, a small proportion of the goat anti-idiotypic antibodies can also interact with the formyl peptide receptor on the neutrophil. The latter conclusion is based on the following evidence. First, direct binding of anti-idiotypic IgG to the PMN did occur, even though high concentrations of IgG were required before a significant increase in binding over preimmune controls was detected (Fig. 6). Second, $F(ab')_2$ fragments of goat anti-idiotype could partially inhibit the binding of f Met-Leu-(^{3}H) Phe to rabbit neutrophils, whereas $F(ab')_2$ fragments from preimmune IgG had no effect (Fig. 5). The simplest reason for the only partial inhibition of f Met-Leu(^{3}H)Phe binding is the low concentration of anti-formyl peptide receptor antibody in the anti-idiotypic antiserum, although if the antibody were of low affinity it would have the same effect. The fact that no plateau of binding was reached even at the highest concentrations tested is compatible with both explanations, which are, of course, not mutally exclusive. Alternatively, these data are also compatible with recent evidence that several forms of the formyl peptide receptor may actually exist (21). Third, absorption of anti-idiotypic activity by passing the goat anti-idiotype IgG through an anti-f Met-Leu-Phe immunoabsorbent column removed the anti-neutrophil formylpeptide receptor activity as determined by inhibition of f Met-Leu-(^{3}H)Phe binding to PMN (Fig. 7). This finding clearly shows that the anti-f Met-Leu-Phe binds this subpopulation of anti-idiotypic antibody. However, selective removal and enrichment of this subpopulation has not been accomplished. Rabbit and/or rat anti-f Met-leu-Phe antibodies other than the one used for anti-idiotype production were not tested in this regard. Preliminary attempts to show depletion of anti-idiotypic binding activity by absorption with rabbit polymorphonuclear leukocytes failed, presumably because of the small proportion of the anti-idiotypic antibody involved.

Several laboratories have shown that anti-idiotypic antibodies raised against antibodies to ligands that react with cellular receptors also react with the corresponding cell surface receptors (22–25). In some instances, these anti-idiotypic antibodies were able to mimic the biologic responses caused by the original ligand. We were unable to demonstrate that our goat anti-idiotypic antibody has analogous biologic activity because the preimmune control IgG preparations induced locomotion, and in the presence or absence of cylochalasin B, granule enzyme release. These "nonspecific" effects probably reflect the "leukoaggressin" activity that Hayashi and co-workers (26) have demonstrated in the large proteolytic fragments of IgG. This problem is being investigated.

At least two explanations are possible for the apparent intra- and inter-species idiotypic cross-reactivity of anti-f Met-Leu-Phe antibodies with the goat anti-idiotopic IgG and the further ability of the latter to react with the neutrophil formylpeptide receptor. First, it is possible that there are ligand recognition sites (idiotopes) in part of the neutrophil formylpeptide receptor and in the rabbit and rat anti-f Met-Leu-Phe antibodies that are structurally or conformationally similar. Antibodies raised against these common idiotopes would allow cross-reactivity between the antibody and the receptor wherever found. Consonant with this hypothesis is our finding, described in the accompanying report (1), that the specificity of the anti-f Met-Leu-Phe binding site is very similar to that of the formyl-peptide neutrophil receptor. However, similarity of specificity among antibodies does not, in and of itself, suggest that the corresponding idiotypes will cross-react. The original observation (27), repeatedly confirmed, showed that the incidence of cross-reaction was low, although numerous exceptions have been found (8).

An alternative explanation is one based on Jerne's concept of "internal image" (28). Jerne proposed that the immune system is regulated by a network comprised of idiotype and anti-idiotype interactions. In some cases a subpopulation of anti-idiotypic antibodies can possess an epitope in common with the foreign antigen and thus be viewed as containing a positive imprint of the foreign antigen. These anti-idiotypic antibodies have been termed internal images by Jerne (28) and "homobodies" by Lindenmann (29). In this view, the extensive cross-reactivity we have described could be due to the presence in low concentrations of a unique subpopulation of anti-idiotypic "internal image" antibodies that possess an epitope in common with f Met-Leu-Phe, i.e., the putative "internal image" subset of anti-idiotype may contain in its variable region Met-Leu-Phe or an homologous sequence or an antigenically similar epitope. This homology may enable the anti-idiotypic antibody to partially mimic the binding activities of f Met-Leu-Phe to the neutrophil and antibody receptors.

Several experimental findings favor this explanation. First is the low percentage of inhibition in the anti-idiotype reactions for both rabbit and rat anti-f Met-Leu-Phe antibodies and the low concentration of anti-idiotype binding directly to the neutrophil. Second, the ability to block idiotype-anti-idiotype binding by the several rat anti-f Met-Leu-Phe antibodies appeared to parallel their antibody binding titers for f Met-Leu-(^{3}H)Phe (10). In addition, the rank order with which the several rabbit

anti-f Met-Leu-Phe antibodies bound goat anti-idiotypic IgG also paralleled their anti-f Met-Leu-Phe titers (10). Lastly, this explanation does not require a common genetic origin for any of the receptors involved.

Despite the uncertainties as to the nature and origin of the cross-reactive anti-idiotypic antibodies, our findings, together with those in the literature, suggest that obtaining anti-idiotypic antibodies to antibodies to cellular ligands may be a general procedure for obtaining antibodies to the corresponding cellular receptors, thus obviating the difficult and laborious chore of first purifying the receptor. We have also produced similar anti-idiotypic antisera in mice, and it may be possible to combine their production in this species with the hybridoma technique to produce high-titer antibody to cellular receptors. This combined procedure may be somewhat more convenient and more certain than directly using the hybridoma technique to prepare anti-cellular antibody (30) with the attendant difficulty of "fishing out" a single anti-receptor clone from a large number of clones producing antibodies against other cellular components.

There are other possible implications of this work. The proposal that "internal image" anti-idiotypic IgG can also explain the apparent cross-reactive idiotypes implies that under these circumstances no necessary genetic relationship is present among the idiotypic molecules involved. This, idiotypic cross-reactions associated with a common genetic origin must be interpreted with caution.

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