mine synthesis in caudate nuclei lesioned with kainic acid indicates that neither postsynaptic dopamine receptors nor the integrity of a striato-nigral feedback loop are essential for this action of enkephalins.

Since, in the rat, the caudate nucleus contains the largest number of opiate receptors (*1*), the opiate receptors are associated with synaptosomes (*16*), and lesion of the nigro-striatal dopaminergic pathway with 6-hydroxy-dopamine eliminates the high-affinity binding for dihydromorphine in the caudate nucleus (*17*), it is conceivable that the narcotic receptors responsible for the above effect are located presynaptically on dopamine nerve terminals.

The mechanism by which enkephalins stimulate dopamine synthesis is not known. It is possible that activation of opioid receptors at the dopamine nerve terminals results in a decreased release of dopamine which, in turn, increases dopamine synthesis because of removal of the normal inhibitory effect of dopamine on presynaptic receptors (*18*).

Consistent with this hypothesis is the report of Loh *et al.* (*19*) that morphine and β-endorphine inhibit potassium chloride–induced release of dopamine from striatal synaptosomes in vitro, an effect which is antagonized by naloxone. On the other hand, an inhibition of dopamine release might explain the cataleptic response to enkephalins (*5a*).

Our data indicate that the activation of narcotic receptors by the naturally occurring methionine-enkephalin may have a physiological role in controlling dopaminergic activity.

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Chemotactic Antibody

Abstract. *Antibody of the immunoglobulin G class to herpes simplex virus and antibody of the immunoglobulin M class to sheep red blood cells were coupled to the synthetic peptide formylmethionylleucylphenylalanine (fMet-Leu-Phe), which is chemotactic for both mononuclear and polymorphonuclear leukocytes. The resulting molecules were chemotactic and retained their antigen-binding activity. When antibodies coupled to fMet-Leu-Phe were incubated with antigen, the resulting immune complexes were also chemotactic. Chemotactic antibody may provide a potent means of enhancing the migration of inflammatory cells to specific sites.*

Inflammation is a common host response to a variety of disease processes. In recent years, a number of naturally occurring biological mediators that cause leukocytes to migrate have been identified (*1*). The resulting accumulation of leukocytes plays an important role in the defense against bacteria, viruses, and tumors. A deficiency in chemotactic factors generated locally may be the cause of inadequate numbers of leukocytes at the site of a lesion, resulting in failure to cope with certain infections or tumors.

A group of synthetic formylated (f) peptides of low molecular weight has been found to be chemotactic at concentrations of 10^{-6} to $10^{-11}M$ (*2*). One of the most active of these peptides is formylmethionylleucylphenylalanine (fMet-Leu-Phe) (*3*). If this small peptide could be coupled to antibody directed against a specific virus or tumor, the resulting

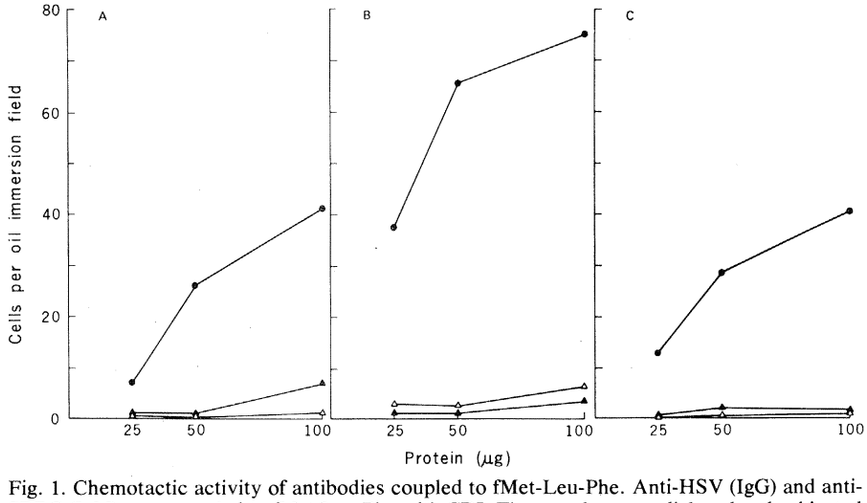

Fig. 1. Chemotactic activity of antibodies coupled to fMet-Leu-Phe. Anti-HSV (IgG) and anti-SRBC (IgM) were coupled to fMet-Leu-Phe with CDI. The samples were dialyzed and subjected to molecular sieve chromatography. The antibody-containing peaks were concentrated and assayed for chemotactic activity using guinea pig peritoneal macrophages. (A) (●—●) Anti-HSV coupled to fMet-Leu-Phe with CDI; (△—△) anti-HSV treated with CDI; (▲—▲) untreated anti-HSV. (B) (●—●) Anti-SRBC coupled to fMet-Leu-Phe with CDI; (△—△) anti-SRBC treated with CDI; (▲—▲) untreated anti-SRBC. (C) Chemotactic activity of immune complexes containing anti-SRBC and 0.083 mg of nitrogen from SRBC stroma. (●—●) Anti-SRBC coupled to fMet-Leu-Phe with CDI; (△—△) anti-SRBC treated with CDI; (▲—▲) untreated anti-SRBC. Each point represents the mean of duplicate samples.

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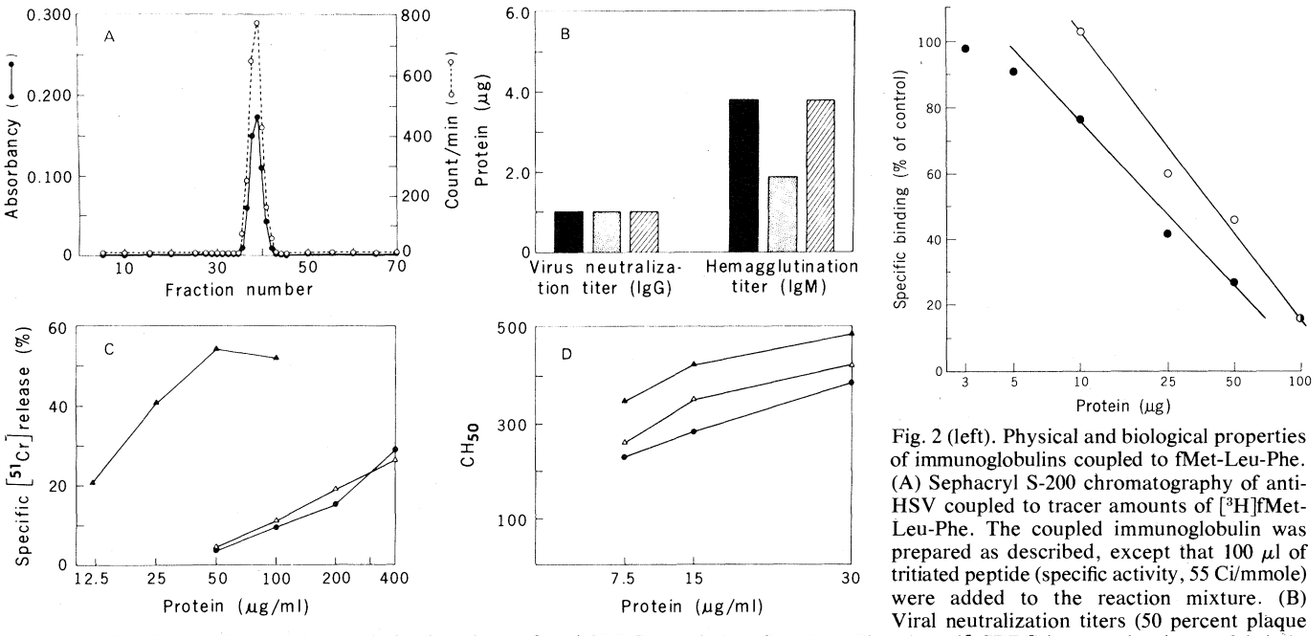

Fig. 2 (left). Physical and biological properties of immunoglobulins coupled to fMet-Leu-Phe. (A) Sephacryl S-200 chromatography of anti-HSV coupled to tracer amounts of [^{3}H]fMet-Leu-Phe. The coupled immunoglobulin was prepared as described, except that 100 μl of tritiated peptide (specific activity, 55 Ci/mmole) were added to the reaction mixture. (B) Viral neutralization titers (50 percent plaque reduction) (*9*) of anti-HSV and hemagglutination titers of anti-SRBC coupled to fMet-Leu-Phe (4×10^7 SRBC in a total volume of 0.6ml). (■) Antibody coupled to fMet-Leu-Phe with CDI; (□) antibody treated with CDI; (▨) untreated antibody. (C and D) Complement-dependent lysis mediated by IgG and IgM immunoglobulins coupled to fMet-Leu-Phe. (C) Suspensions of HSV-infected primary rabbit kidney cells labeled with ^{51}Cr were incubated with anti-HSV plus complement and ^{51}Cr release determined (*10*). Percent specific ^{51}Cr release was calculated (*11*). (●—●) Anti-HSV coupled to fMet-Leu-Phe with CDI; (△—△) anti-HSV treated with CDI; (▲—▲) untreated anti-HSV. (D) Hemolytic titrations of anti-SRBC were carried out by standard methods and 50 percent hemolytic units (CH_{50}) determined (*8*, p. 149). (●—●) Anti-SRBC coupled to fMet-Leu-Phe with CDI; (△—△) anti-SRBC treated with CDI; (▲—▲) untreated anti-SRBC. Fig. 3 (top right). Binding to rabbit neutrophils of IgG and IgM antibodies coupled to fMet-Leu-Phe. The coupled antibodies were assayed for their capacity to inhibit the binding of [^{3}H]fNle-Leu-Phe to neutrophils by methods previously described (*7*). (●—●) Anti-HSV coupled to fMet-Leu-Phe with CDI; (○—○) anti-SRBC coupled to fMet-Leu-Phe with CDI.

molecule might be capable of binding to target tissue where it also might attract inflammatory cells. The experiments reported here describe the coupling of fMet-Leu-Phe to immunoglobulins of the G and M (IgG and IgM) classes to form antibody molecules with chemotactic activity.

Rabbit IgG directed against herpes simplex virus type 1 (HSV) (anti-HSV) was obtained by precipitation of the serum with 50 percent ammonium sulfate, DE-52 cellulose (Whatman) chromatography (0.01*M* phosphate buffer, *p*H 8.0), concentration by ultrafiltration, and dialysis against tris-NaCl buffer (0.05*M* tris, 0.1*M* NaCl, *p*H 7.5). The IgM fraction of rabbit antiserum against sheep red blood cells (SRBC) (anti-SRBC) (Flow Laboratories) was obtained by Sephacryl S-200 and Sepharose 4B-Cl (Pharmacia) chromatography with tris-NaCl buffer. Both antibodies were coupled to fMet-Leu-Phe by 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (CDI)(Aldrich Chemical) (*4*) in a weight ratio of 3.6 parts of antibody to 1.0 part of peptide to 36.3 parts of CDI. The coupled IgG and IgM antibodies were dialyzed against tris-NaCl buffer and chromatographed on Sephacryl S-200 or Sepharose 4B-Cl, respectively. Identical procedures were performed on controls including native antibody and antibody treated only with CDI. Peaks eluting in the region of the native immunoglobulins were concentrated by ultrafiltration, and chemotaxis assays were performed (*5*).

Antibodies can acquire chemotactic activity by covalent coupling with the synthetic chemotactic peptide, fMet-Leu-Phe (Fig. 1). Immunoglobulins of both the IgG (Fig. 1A) and the IgM (Fig. 1B) classes chemically modified in this manner attract macrophages in vitro. Migration of these cells was induced by 50 to 100 μg of protein of either preparation. The unmodified IgG and IgM immunoglobulins do not possess chemotactic activity nor do they acquire this activity when treated with the coupling agent CDI. In additional studies, polymorphonuclear leukocytes also responded to these chemotactic immunoglobulin preparations (data not shown).

Some of the physical and biological properties of immunoglobulin coupled to the chemotactic peptide are illustrated in Fig. 2. Anti-HSV (IgG) coupled with tracer amounts of tritiated fMet-Leu-Phe (New England Nuclear) was chromatographed on Sephacryl S-200. As shown in Fig. 2A, the peak of radioactivity coincided exactly with the IgG peak. From these data and Lowry protein determinations, it was calculated that approximately ten peptide molecules were linked to each immunoglobulin molecule. Immunoglobulins coupled to the chemotactic peptide retained their reactivity with antigen (Fig. 2B). The coupled anti-HSV was as effective in neutralizing virus as native antibody or antibody treated with CDI alone. Similarly, coupling the peptide to anti-SRBC (IgM) did not result in loss of hemagglutinating activity. Although no adverse effect on antigen binding occurred after coupling of this peptide to either of these immunoglobulins, the anti-HSV showed a marked reduction in complement-dependent lysis, as determined by ^{51}Cr release from HSV-infected cells (Fig. 2C). However, this loss in activity was not caused by the presence of the peptide since CDI treatment alone produced the identical effect. In contrast, the hemolytic activity of the anti-SRBC was only slightly decreased by coupling it to the peptide (Fig. 2D). This suggests that CDI treatment may alter the tertiary structure of IgG but not IgM molecules.

Since synthetic chemotactic peptides bind receptors on the surface of polymorphonuclear and mononuclear leukocytes (*6*, *7*), the capacity of the coupled fMet-Leu-Phe to inhibit the binding of free tritiated peptide was examined. The

binding curves (Fig. 3) show that fMet-Leu-Phe coupled to IgG and IgM antibodies inhibited the binding of tritiated f-norleucylleucylphenylalanine (fNle-Leu-Phe) (*3*) by 50 percent at 22 μg and 40 μg, respectively. Neither the native immunoglobulins nor those treated only with CDI (up to 150 μg) inhibited binding (data not shown). These studies demonstrate that the chemotactic peptide, although linked to immunoglobulins, can still bind its cellular receptors.

Since the coupled immunoglobulins possessed potent chemotactic activity as well as antigen binding activity, studies were carried out to determine whether these preparations retained their chemotactic activity when complexed to antigen. Untreated, CDI-treated, or peptide coupled anti-SRBC were incubated for 1 hour at 37°C with SRBC stroma (*8*, p. 150) and assayed for chemotactic activity. Immune complexes containing the peptide were chemotactic, but immune complexes lacking the peptide were inactive (Fig. 1C). Washing the complexes three times caused no loss in chemotactic activity, indicating that this property resides in the complex and not in free antibody. Moreover, centrifugation to remove complexes produced supernatants completely devoid of chemotactic activity.

The effectiveness of chemotactic antibody in vivo is presently under investigation. In theory, the coupled antibody will reach its target and exert its effect locally by attracting inflammatory cells. Demonstrating that the binding of chemotactic antibody to membranes results in chemotactic immune complexes suggests that such antibody-antigen complexes shed from cells in vivo may also be chemotactic. This might be particularly useful in situations where the foreign antigens (tumors or infected cells) are poor immunostimulants and trigger little if any inflammatory response. If chemotactic antibody proves effective in vivo, it should be possible to couple a variety of other biologically active mediators to immunoglobulin molecules.

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Tumor Promoters Inhibit Morphological Differentiation in Cultured Mouse Neuroblastoma Cells

Abstract. *When added to mouse neuroblastoma cultures, the potent tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) inhibits spontaneous neurite formation as well as that induced in response to serum deprivation, prostaglandin E_1, 5-bromo-2'-deoxyuridine, and papaverine. Other tumor-promoting macrocyclic plant diterpenes also inhibit neurite formation, whereas nonpromoting diterpenes do not. Inhibition by TPA was reversible and was unrelated to toxicity.*

The mouse neuroblastoma cell lines initially established in culture from the C1300 solid tumor (*1*) can be used to study many aspects of neuronal differentiation. These cells contain neural enzymes (*1–3*), produce catecholamines (*2*), possess the neuron-specific 14-3-2 protein (*4*), and are electrically excitable (*5*). Cells of both subcutaneous tumors and suspension cultures in vitro are anaplastic and round. When, however, they are grown in culture attached to a surface and in the presence of dibutyryl adenosine 3',5'-monophosphate (cyclic AMP) (*6*), bromodeoxyuridine (*7*), prostaglandin E_1 (*8*), cytosine arabinoside (*9*), papaverine (*10*), or serum-free medium (*11, 12*) the cells assume a morphology resembling the mature neuron (*2*). In particular, neurites are extended which contain microtubules, neurofilaments, and dense core vesicles. The development of neurites by these cells facilitates studies of morphological differentiation.

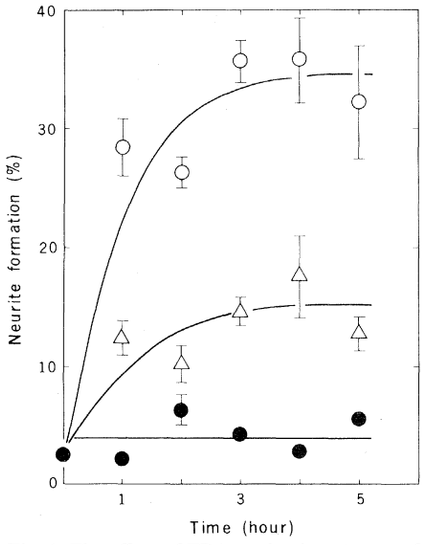

Fig. 1. The effect of TPA on the time course of neurite outgrowth in murine neuroblastoma cells during serum deprivation. The cells were incubated for up to 5 hours in DMEM (*25*) in the presence of serum (●), absence of serum (○), or absence of serum plus 100 ng of TPA per milliliter (△).

We have studied the effects of 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) on morphologic differentiation of murine neuroblastoma cultures. This compound and other plant diterpenes are among a class of potent tumor-promoting agents in the two-stage mouse skin carcinogenesis system (*13–15*). Effects of TPA on macromolecular synthesis (*16*), phospholipid metabolism (*17*), and alterations in ultrastructure (*18*) in mouse skin have been reported. Also, TPA induces a number of alterations in cultures of normal cells which tend to mimic the phenotype of cells transformed by viruses (*19, 20*) or chemical carcinogens (*20*), including induction of the protease plasminogen activator (*21*). Recently, it has been shown that TPA can inhibit differentiation in murine erythroleukemia and chicken myoblast cultures (*22–24*). Here we report that TPA causes a reversible inhibition of the program of differentiation in neuroblastoma cultures.

Murine neuroblastoma cells (clone Neuro 2a) were obtained from the American Type Culture Collection (ATCC

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