Immunology 1990 **71** 52–56

Substance P production by P388D1 macrophages: a possible autocrine function for this neuropeptide

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Accepted for publication 22 May 1990

SUMMARY

This study investigates the ability of P388D1 macrophages to synthesize and secrete substance P (SP). Using a monoclonal anti-SP antibody (termed MASP-1) coupled to Sepharose, it was possible to immunoaffinity purify from culture supernates a peptide that was antigenically related to SP. P388D1 macrophage cultured in the presence of ^{35}S-methionine secreted into culture supernates a labelled peptide which could be recognized by MASP-1. Affinity-purified, P388D1-derived SP was shown to be chemically similar to synthetic SP using gel-filtration chromatography and reverse-phase HPLC. In addition, an RIA using a polyclonal, monospecific antibody was used to quantify the amount of secreted SP in cultured supernates. P388D1 macrophages secreted 222 pg SP per 10^8 cells, whereas SP secretion by control thymocyte cultures was not detectable. The functionality of the P388D1-derived SP was also investigated. Since exogenously added SP can increase secretion of an interleukin-1 (IL-1)-like activity in these cells, we questioned whether an anti-SP antibody could remove P388D1-secreted SP from the culture, and in turn reduce cytokine production. By culturing varying dilutions of MASP-1 with P388D1 cells, it was possible to decrease cytokine production by P388D1 cells compared to cultures containing no antibody or with normal mouse immunoglobuln G (IgG). Taken together, these studies demonstrate that macrophage-derived SP may function in an autocrine or paracrine fashion to modulate macrophage function.

INTRODUCTION

In vitro cell cultures have been utilized to demonstrate a variety of effects of substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) on cells of the immune system (reviewed by Payan, 1989). Included in these effects are increased immunoglobulin secretion by B lymphocytes, increased proliferation of T lymphocytes and increased histamine release from mast cells. Recently, it was shown that substance P (SP) could induce the release of interleukin-1 (IL-1)-like activity from the mouse macrophage cell line P388D1 (Kimball, Persico & Vaught, 1988), and could also induce human peripheral blood monocytes to release IL-1, IL-6, and tumour necrosis necrosis factor-alpha (TNF-α) (Lotz, Vaughan & Carson, 1988). Based on the *in vitro* studies, it has been suggested that SP has tremendous potential to function *in vivo* as an immunomodulator. This possibility is supported by the finding of specific high-affinity receptors for SP on lymphocytes (reviewed by Bost, 1988) and macrophages (Hartung, Wolters & Toyka, 1986). In addition, SP has been detected *in vivo* at locations and in concentrations which would appear to allow interaction of receptor-bearing immunocytes with this neuropeptide (Stead *et al.*, 1987). For example, increased levels of SP have been found in inflammatory exudates from rats (Tissot, Pradelles & Giroud, 1988) and in humans with inflammatory bowel disease (Koch, Carney & Go, 1987). Interestingly, receptor-binding sites for SP were also increased in patients with inflammatory bowel disease (Mantyh *et al.*, 1988).

The presence of SP in inflammatory exudates has been attributed to the secretion of this neuropeptide from peripheral neurons (Nakanishi, 1987). Recently, eosinophils have been shown to synthesize immunoreactive SP (Aliakbari *et al.*, 1987) and also to express a mRNA encoding SP (Weinstock *et al.*, 1988). Therefore, SP-containing neurons may not be the only source of this neuropeptide for immune responses such as inflammation. This report focuses on the ability of another type of immunocyte (i.e. macrophages) to synthesize and secrete SP. The results presented here show that P388D1 macrophages produce SP and respond to that production in an autocrine or paracrine-like fashion.

Abbreviations: IL-1, interleukin-1; IL-6, interleukin-6; MASP-1, monoclonal anti-SP antibody; SP, substance P.

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MATERIALS AND METHODS

Cell lines

The murine macrophage cell line P388D1, from ATCC (TIB 63), was propagated in RPMI-1640 supplemented with 1% non-

essential amino acids, 1% glutamine, 1% sodium pyruvate, 1% penicillin-streptomycin (Gibco, Grand Island, NY) and 10% foetal calf serum (FCS; Hyclone, Logan, UT). The endotoxin levels in culture media were less than 0·1 ng/ml, as determined using a Limulus amoebocyte assay (Sigma, St Louis, MO).

Peptides

Synthetic substance P (SP) and methionine-enkephalin were purchased from Sigma Chemicals, and SP (4-11) from Peninsula Laboratories (Belmont, CA). Stock solutions (0·1 mM) for each peptide were prepared by initially dissolving lyophilized peptides in 0·2 M acetic acid, pH 4·0 and subsequently diluting in phosphate-buffered saline (PBS), pH 7·2, and stored at −20°. Oxidized SP was prepared by the method described by Savige & Fontana (1977). Adrenocorticotropin (ACTH) was prepared and purified as described previously (Clarke & Bost, 1989).

Preparation of a monoclonal anti-SP antibody

SP coupled to keyhole limpet haemocyanin (Pascual & Bost, 1989) was injected into BALB/c mice. When anti-SP titres were greater than 1:100,000, mononuclear splenocytes were fused with SP2/0 myeloma cells, from ATCC (CRL 1581), using polyethylene glycol (Boehringer-Mannheim, Indianapolis, IN) as described previously (Carr, Blalock & Bost, 1989). Supernates from fusion wells were subsequently screened for reactivity against SP using an ELISA procedure, as described previously (Pascual & Bost, 1989). Several positive wells were selected, expanded and cloned in soft agar. Ascitic fluid from one cloned hybrid (termed MASP-1) was produced in pristane-primed BALB/c mice. Anitibody was purified by passage over a protein G-conjugated affinity column (Genex, Gaithersburg, MD) and reactivity of the antibody with various peptides was determined using a radioimmunoassay (RIA). Relative to SP, reactivity with SP (1-6) was 62%; SP (1-4) <0·01%; SP (6-11) <0·01%; substance K (SK) <0·01%; and eledoisin <0·01%. Thus, it is clear that the monoclonal antibody, (mAb) MASP-1, recognizes the amino terminal portion of SP.

^{35}S-methionine labelling and immunoaffinity chromatography

FCS was dialysed in 1000 MW exclusion dialysis tubing against RPMI-1640 without methionine (selectamine kit; Gibco) to reduce the amount of free methionine. Twenty million P388D1 cells were cultured in 10 ml methionine minus RPMI-1640 containing 4% dialysed FCS and 25–50 μCi ^{35}S-methionine (New England Nuclear, Boston, MA). After 48 hr, supernates from these cultures were filtered and passed over an immunoaffinity column prepared by coupling the MASP-1 mAb to cyanogen-activated Sepharose CL-4B (Sigma) (Cuatrecasas, Wilchek & Anfinsen, 1968). Supernatant material not adhering to the affinity column was washed through with PBS (0·15 M NaCl, 0·05 M phosphate, pH 7·2). Material adhering to the column was eluted with 0·1 M acetic acid, pH 3·2, and lyophilized prior to characterization using gel-filtration chromatography or high-pressure liquid chromatography (HPLC).

Gel filtration chromatography

The relative elution volumes of a variety of standard peptides were determined using a P4 (Bio-Rad, Rockville Centre, NY) gel-filtration column (1·8 cm × 46 cm) equilibrated in 0·1 M acetic acid, 0·15 M NaCl. Material from P388D1 culture supernates adhering to the immunoaffinity column was lyophilized, reconstituted in 0·1 M actic acid, 0·15 M NaCl and applied to the gel-filtration column. Fractions were counted for the presence of ^{35}S-methionine using a scintillation cocktail (ICN, Irvine, CA) compatible with aqueous samples.

HPLC

Material from P388D1 cultural supernates adhering to the immunoaffinity column was lymphilized, reconstituted in 10% acetonitrile, and applied to a semi-preparative, 300A, C-18 HPLC column (Rainin, Woburn, MA). A 60-min 10–60% acetonitrile/H_2O gradient was used. Fractions were collected and counted for the presence of ^{35}S-methionine using a scintillation cocktail compatible with aqueous samples.

SP RIA

An RIA was developed using a commercially available rabbit anti-SP antisera (Peninsula Laboratories). This antisera was specific for SP in that it reacted less than 1% with endorphin, enkephalin, ACTH, somatostatin, vasopressin or SK. P388D1 (5×10^7 cells) were cultured for 3 days in supplemented RPMI-1640 and 5% FCS. Cells were separated and the supernates were subsequently lyophilized and resuspended with deionized, sterile water at 1/10 the initial volume. Twenty-thousand c.p.m. ^{125}I-Tyr^8-SP (2200 Ci/mmole; New England Nuclear) were added to microfuge tubes containing 50 μl anti-SP antisera and 100 μl culture supernate or 100 μl of media containing varying amounts of unlabelled SP. After an overnight incubation, 1·0 mg of anti-rabbit IgG-conjugated microspheres (KPL, Gaithersburg, MD) were added to each tube for 18 hr. After washing twice by centrifugation, microspheres were counted for the presence of radioactivity. The concentration of SP present was determined by the ability to block ^{125}I-Tyr^8-SP binding. The detection limit of this assay was approximately 2 pg SP.

Thymocyte proliferation assay

A thymocyte proliferation assay (Larrick, Brindley & Doyle, 1985) was used to detect the presence of cytokine activity in culture supernates of P388D1 cells. While this assay has been routinely used in the past to detect IL-1, it has recently been demonstrated that IL-6 will also stimulate the proliferation of thymocytes in this assay (Suda *et al.*, 1989). Therefore the assay used did not distinguish between IL-1 or IL-6; however, the purpose of these experiments was to demonstrate an effect of P388D1-secreted SP on cytokine production by these cells. To this end, 5×10^5 P388D1 cells were cultured in 1 ml supplemented RPMI-1640 (no FCS) for 18 hr with or without varying concentrations of MASP-1 or normal mouse IgG (Sigma). Supernates were harvested, diluted 1:20, and added to cultures of 5×10^5 BALB/c thymocytes in RPMI-1640 with 10% FCS and 1 μg/ml concanavalin A (Sigma). Thymocytes were cultured for 72 hr with the last 4 hr being in the presence of 0·5 μCi tritiated thymidine (New England Nuclear). Cells were harvested onto glass fibre filter (Mini-mash; M.A. Bioproducts, Walkersville, MD), and counted for the presence of tritium.

RESULTS

Immunoaffinity purification of ^{35}S-methionine-labelled SP

P388D1 macrophages were cultured for 48 hr with ^{35}S-methionine in RPMI-1640 depleted of excess methionine. Supernate

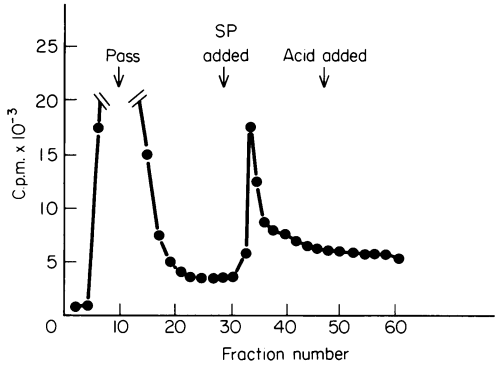

Figure 1. Immunoaffinity purification of P388D1-secreted SP. P388D1 cells were cultured in the presence of ^{35}S-methionine. Supernates from these cultures were passed over an immunoaffinity column conjugated with a monoclonal anti-SP antibody, as indicated by the arrow. Adherent material was eluted by adding 1·0 μM synthetic SP, as indicated by the arrow. To assure that all bound material had been eluted from the column, 0·1 M acetic acid (pH 3·0) was added, as indicated by the arrow. Fractions were collected and the amount of radioactivity in each fraction was determined using a scintillation cocktail compatible with aqueous samples.

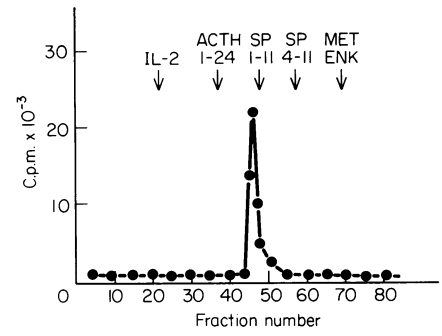

Figure 2. Gel-filtration chromatography of affinity-purified P388D1-derived SP. Supernates from ^{35}S-methionine-treated P388D1 cultures were passed over an anti-SP affinity column, as described in Fig. 1. Material eluted from the immunoaffinity column was applied to a P4 gel filtration column. Fractions were collected and the amount of radioactivity in each fraction was determined using a scintillation cocktail compatible with aqueous samples. For comparison, the elution volumes of standard peptides are shown with arrows. Standards included adrenocorticotropin (ACTH 1-24), SP 1-11, SP 4-11 and methionine-enkephalin (MET ENK).

from the culture was then passed over an immunoaffinity column conjugated with a monoclonal anti-SP antibody, termed MASP-1. Figure 1 depicts a typical immunoaffinity purification profile. After washing unbound ^{35}S-methionine through the MASP-1 affinity column, unlabelled SP at a concentration of 1·0 μM eluted a peak of radioactivity from the column. The unlabelled SP appeared to elute essentially all of the bound radioactivity since very little radioactivity could be eluted using a subsequent acid wash. Considering the specificity of the antibody and the selectivity of the elution conditions, it seemed likely that the P388D1 macrophage supernate contained a molecule which was antigenically similar to SP. Furthermore, it was obvious that the molecule adhering to the affinty column had been synthesized and secreted by the P388D1 macrophages during culture, since it had been labelled with ^{35}S-methionine. The fact that methionine was incorporated suggested the material was protein or peptide in nature.

Characterization of the immunoaffinity-purified material

In an effort to determine if the material secreted by P388D1 macrophages and binding the affinity column was in fact SP, relative elution volumes from gel-filtration and C-18 HPLC columns were determined. Figure 2 is a P4 chromatographic profile of affinity-purified material from the supernates of P388D1 macrophages. For comparison, the sizing column was marked with peptides of varying lengths, and their elution volumes are indicated in Fig. 2. Clearly the affinity-purified material had an elution volume almost identical to that of synthetic SP, suggesting that these two molecules were similar in size.

Affinity-purified material was also subjected to reverse-phase HPLC using a C-18 column. As shown in Fig. 3, the majority of radiolabel eluted just prior to the unlabelled SP used as a standard. Initially, this result was troublesome since it was clear that most of the affinity-purified material did not co-elute with SP. In retrospect, the methods used to isolate and concentrate the radiolabelled material were ones which have

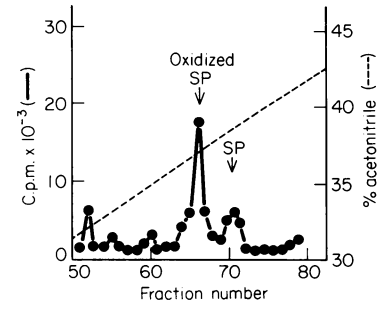

Figure 3. Reverse-phase HPLC of immunoaffinity-purified P388D1-derived SP. Supernates from ^{35}S-methionine-treated P388D1 cultures were passed over an anti-SP affinity column, as described in Fig. 1. Using a C-18 HPLC column, immunoaffinity-purified material was eluted in an acetonitrile/water gradient. Fractions were collected and the amount of radioactivity determined using a scintillation cocktail compatible with aqueous samples. The solid line represents counts per minute, while the dashed line indicates the percentage of acetonitrile. For comparison, the elution volumes of synthetic SP and synthetic SP that was exposed to oxidizing conditions are shown.

been reported to oxidize SP (Floor & Leeman, 1980). Furthermore, it has been shown that oxidized SP elutes just prior to SP on reverse-phase HPLC (Foy *et al.*, 1989). Therefore, when oxidized SP was used to mark the column, it co-eluted with the radiolabelled material, suggesting that our isolation procedure had oxidized this peptide.

Quantification of SP secreted into culture supernates by P388D1 macrophages

In an effort to quantify the amount of SP in supernates of P388D1 macrophage cultures, an RIA was used. The anti-SP antibody used in this assay was obtained commercially and, while being polyclonal, was monospecific for SP. As shown in Fig. 4, when varying concentrations of P388D1 macrophage

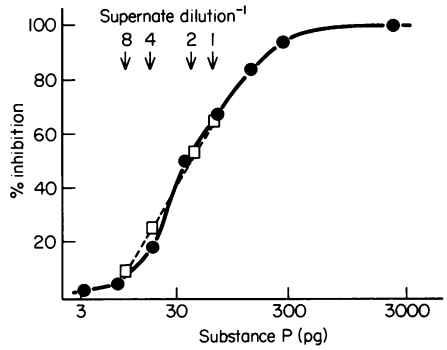

Figure 4. RIA of SP in P388D1 culture supernates. An RIA for SP was developed using a polyclonal, monospecific anti-SP antibody, which bound ^{125}I-Tyr^{8}-SP. To generate a standard inhibition curve, varying concentrations of synthetic SP (●) were used to block binding. In addition, varying dilutions of P388D1 culture supernates (□) were also assayed for their ability to block ^{125}I-Tyr^{8}-SP binding to the antibody. Results are presented as means of duplicate determinations, with SD always being less than 10% of the mean values.

Table 1. Quantity of SP in supernate of cultured P388D1 macrophages

Cultured cells	pg SP/10^{8} cells
P388D1 mouse macrophages	222·5
Rat thymocytes	<2·0

Cells were cultured for 3 days prior to harvesting and concentrating the supernates. The amount of SP in supernates was calculated using an RIA (Fig. 4) with a sensitivity of approximately 2·0 pg SP. Values are reported as means of triplicate determinations, with SD always being less than 10% of the mean. These experiments were performed three times with similar results.

supernates were assayed for their ability to block ^{125}I-Tyr^{8}-SP binding to anti-SP antibodies, the inhibition was dose-dependent and followed the slope of a standard inhibition curve. Parallel inhibition curves for varying supernate dilutions suggested again that P388D1 macrophages were secreting authentic SP.

Using the same RIA, the amount of P388D1 macrophage-derived SP secreted into culture supernates was quantified and is shown in Table 1. Compared to rat thymocyte cultures, which served as a negative control, significant levels of SP were detected in supernates from P388D1 macrophage cultures. In fact, 222·5 pg SP per 10^{8} cells were detected in P388D1 culture supernates, whereas essentially no SP could be detected in supernates of thymocyte cultures.

Inhibition of cytokine production by P388D1 macrophages using a monoclonal anti-SP antibody

P388D1 macrophages can be stimulated by SP to secrete cytokine(s) which have IL-1-like activity in a thymocyte proliferation assay (Kimball *et al.*, 1988). We questioned whether the SP secreted by these cells might be, in part, responsible for increasing the production of these cytokines in an autocrine or paracrine fashion. To address this question, cultures of P388D1

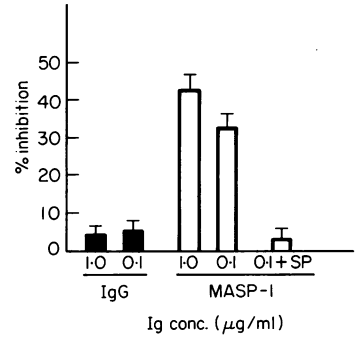

Figure 5. Inhibition of cytokine production by P388D1 macrophages using a monoclonal anti-SP antibody. Cultures of P388D1 cells were incubated with control mouse IgG (solid bars) or a monoclonal anti-SP antibody, termed MASP-1 (open bars), for 18 hr. Supernates were collected, diluted 1:20 and assayed for IL-1-like activity using a thymocyte proliferation assay. The thymocyte proliferation assay involved incubating thymocytes with supernates in the presence of a suboptimal mitogen concentration (i.e. concanavalin A at 1 μg/ml). Results are presented as percentage inhibition of tritiated thymidine uptake by thymocytes exposed to normal IgG or MASP-1 using triplicate determinations ± SD. To show specificity of the inhibition by MASP-1, 1·0 nM synthetic SP added to the P388D1 cultures was able to block the inhibition. Thymocytes treated with a 1:20 dilution of P388D1 supernate and concanavalin A incorporated 18,347 (±1024) c.p.m. tritiated thymidine. Percentage inhibition was calculated using this value.

cells were incubated with the monoclonal anti-SP antibody, MASP-1, or normal mouse IgG. After 18 hr, supernates were assayed for the presence of IL-1-like activity using a thymocyte proliferation assay. As shown in Fig. 5, when compared with control IgG-treated cultures, MASP-1 reduced by almost 50% the proliferation of thymocytes induced by P388D1 supernates. Presumably, MASP-1 bound the constitutively produced SP, thus limiting the amount of SP available for receptor binding. As a result, a reduction in the IL-1-like activity was observed. Furthermore, this reduction in thymocyte proliferation induced by MASP-1 could be reversed upon addition of synthetic SP (1·0 nM) to P388D1 cultures treated with MASP-1 (Fig. 5). It should be noted that exogenously added SP has no direct effect on thymocyte proliferation (D.W. Pascual and K.L. Bost, unpublished observation). Since MASP-1 does not recognize IL-1 or IL-6 or significantly affect the ability of P388D1 macrophages to proliferate using these culture conditions (data not shown), it is likely that the differences observed here are due to MASP-1 binding SP. Because IL-1 or IL-6 function in a stimulatory fashion in this assay (Suda *et al.*, 1989), it is not clear if one or both of these interleukins is responsible for thymocyte proliferation. Ongoing investigations will allow us to determine which interleukin predominates. Regardless of the outcome of these experiments, we were able to conclude that P388D1-secreted SP can function in an autocrine or paracrine fashion to stimulate cytokine production.

DISCUSSION

These studies demonstrate that P388D1 macrophages have the ability to synthesize and secrete SP. Using a monoclonal anti-SP antibody coupled to Sepharose, material antigenically related to

synthetic SP could be isolated from P388D1 macrophage culture supernates. The incorporation of ^{35}S-methionine into this affinity-purified material not only demonstrated its synthesis by macrophages, but also indicated that the material was either a protein or a peptide. Gel-filtration chromatography, reverse-phase HPLC and a sensitive RIA were criteria used to demonstrate the similarity of P388D1-derived SP to synthetic SP. It should be emphasized that two different monospecific anti-SP antibodies, one polyclonal and one monoclonal, recognized the secreted SP. Thus, in addition to previous reports demonstrating SP production by eosinophils (Aliakbari *et al.*, 1987; Weinstock *et al.*, 1988), it is clear that P388D1 macrophages also possess this ability.

While the concentration of SP detected in P388D1 culture supernates was approximately 20 pM, this does not represent 'total' SP in the culture. Since P388D1 macrophages express SP receptors, receptor-bound peptide would be inaccessible for detection using this RIA. Furthermore, intracellular SP would not be detected since the cells were not lysed prior to RIA, as has been done in previous studies to demonstrate eosinophil-derived SP (Weinstock *et al.*, 1988). Preliminary studies have demonstrated substantial amounts of SP in P388D1 cell lysates (D.W. Pascual and K.L. Bost, unpublished observation), as would be expected. However, the importance of showing a secreted product suggests mechanisms by which this P388D1 macrophage-derived neuropeptide might modulate cell function.

Not only was SP secreted by P388D1 macrophages, but this secreted peptide appears to be functional. The monoclonal anti-SP antibody reduced cytokine production by P388D1 macrophages. This finding suggests a potential autocrine or paracrine function for macrophage-derived SP, since depletion of this neuropeptide decreased cytokine secretion. Speculatively, macrophages activated *in vivo* by an appropriate stimulus may secrete SP into localized areas which could interact with surrounding cells that express SP receptors. Whether or not this scenario is an accurate one will depend upon subsequent studies. The results presented here certainly suggest that it is possible, and that macrophages may be an important source of the immunomodulating peptide SP.

ACKNOWLEDGMENTS

We would like to thank Diane Weigent and Mary Nelle Shelton for the preparation of this manuscript. This work is in part supported by National Heart, Lung, and Blood Institute grants HL07457 and DK39299.

REFERENCES

Aliakbar J., Sreedharan S.P., Turck C.W. & Goetzl E.J. (1987) Selective localizaton of vasoactive intestinal peptide and substance P in human eosinophils. *Biochem. Biophys. Res. Comm.* **148,** 1440.

Bost K.L. (1988) Hormone and neuropeptide receptors on mononuclear leukocytes. *Prog. Allergy*, **43,** 68.

Carr D.J.J, Blalock J.E. & Bost K.L. (1989) Monoclonal antibody against a peptide specified by met-enkephalin complementary RNA recognizes the delta-class opioid receptor. *Immunol. Lett.* **20,** 181.

Clarke B.L. & Bost K.L. (1989) Differential expression of functional adrenocorticotropic hormone receptors by subpopulations of lymphocytes. *J. Immunol.* **143,** 464.

Cuatrecasas P., Wilchek M. & Anfinsen C.B. (1968) Selective enzyme purification by affinity chromatography. *Proc. natl. Acad. Sci. U.S.A.* **61,** 636.

Floor E. & Leeman S.E. (1980) Substance P sulfoxide: separation from substance P by high pressure liquid chromatography, biological and immunological activities, and chemical reduction *Anal. Biochem.* **101,** 498.

Foy W.L., Allen J.M., McKillop J.M., Goldsmith J.P., Johnston C.F. & Buchanan K.D. (1989) Substance P and gastrin releasing peptide in bovine mesenteric lymphatic vessels: chemical characterization and action. *Peptides*, **10,** 533.

Hartung H.P., Wolters K. & Toyka K.V. (1986) Substance P: Binding properties and studies on cellular responses in guinea pig macrophages. *J. Immunol.* **135,** 3856.

Kimball E.S., Persico F.J. & Vaught J.L. (1988) Substance P, neurokinin A, and neurokinin B induce generation of IL-1-like activity in P388D1 cells. *J. Immunol.* **141,** 3564.

Koch T.R., Carney J.A. & Go V.L.W. (1987) Distribution and quantification of gut neuropeptide in normal intestine and inflammatory bowel disease. *Dig. Dis. Sci.* **32,** 369.

Larrick J.W., Brindley L. & Doyle M.V. (1985) An improved assay for detection of interleukin-1. *J. immunol. Meth.* **79,** 39.

Lotz M., Vaughan J.H. & Carson D.A. (1988) Effect of neuropeptides on production of inflammatory cytokines by human monocytes. *Science*, **241,** 1218.

Mantyh C.R., Gates J.S., Zimmerman R.P., Welton M.L., Passard E.P., Vigna S.R., Maggio J.E. Gruger L. & Mantyh P.W. (1988) Receptor binding sites for substance P, but not substance K or neuromedin K are expressed in high concentrations by arterioles, venules, and lymph nodes in surgical specimens obtained from patients with ulcerative colitis and Crohn's disease. *Proc. natl. Acad. Sci. U.S.A.* **85,** 3235.

Nakanishi S. (1987) Substance P precursor and kininogen: their structures, gene organizations, and regulation. *Physiol. Rev.* **67,** 1117.

Pascual D.W. & Bost K.L. (1989) Anti-peptide antibodies recognize anti-substance P antibodies in an idiotypic fashion. *Peptide Res.* **2,** 207.

Payan D.G. (1989) Neuropeptides and inflammation: the role of substance P. *Ann. Rev. Med.* **40,** 341.

Savige W.E. & Fontana A. (1977) Interconversion of methionine and methionine sulfoxide. *Methods Enzymol.* **47,** 453.

Stead R.H., Tomioka M., Quinonez G., Simon G.T., Felten S.Y. & Bienenstock J. (1987) Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc. natl. Acad. Sci. U.S.A.* **84,** 2975.

Suda T., Hodgkin P., Lee, F. & Zlotnik A. (1989) Biological activity of recombinant murine interleukin-6 in interleukin-1 T cell assays. *J. immunol. Meth.* **120,** 173.

Tissot M., Pradelles P. & Giroud J.P. (1988) Substance P-like levels in inflammatory exudates. *Inflam.* **12,** 25.

Weinstock J.V., Blum A., Walder J. & Walder R. (1988) Eosinophils from granulomas in murine *Schistosomiasis mansoni* produce substance P. *J. Immunol.* **141,** 961.