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The nerve growth factor protein, NGF, has been shown to play a physiologic role in the development and regeneration of the peripheral nervous system, acting on sensory and sympathetic ganglia. In the central nervous system, NGF induces choline acetyltransferase in certain cholinergic regions and spares magnocellular neurons following fimbria transections. NGF has been shown to act in vivo on non-neuronal tissues as a modulator of immune and inflammatory reactivity.

We have demonstrated the presence of receptors to NGF on rat and human mononuclear cells and the specific and saturable binding of NGF to these cells. We have also shown that NGF has activating and mitogenic effects on these cells. Our data is consistent with the hypothesis that NGF effects on tissues are important to differentiation of these tissues. Also, that NGF receptors on different tissues are slightly different structurally although the NGF binding properties are very similar.

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Final Report for a Research Project ONR Contract N00014-87-K-0364 Nerve Growth Factor Effects on the Immune System funded by the Office of Naval Research.

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Research Objectives and Approaches.

There is considerable evidence that some regulatory activities of the nervous system and the immune system are jointly regulated through the interactions of specific signal molecules generated by these systems. Neuropeptides whose actions are most often associated with neuronal cell function have more recently been shown to modulate cellular reactivity within the immune system through receptor specific mechanisms. One factor that has regulatory activities within the nervous, endocrine and immune systems is the nerve growth factor protein (NGF). We have demonstrated that functionally active receptors for nerve growth factor (NGFR) are present on cells of immune origin suggesting a possible role for NGF as an immunoregulatory molecule (Thorpe et al, 1987; Thorpe, et al, 1987; Thorpe and Perez-Polo, 1987; Thorpe et al, 1988; Thorpe et al, 1989; Morgan et al, 1989).

Nerve growth factor (NGF) is a protein that is known to be required for the development and maintenance of neurons in the peripheral and central nervous systems as well as chromaffin cells of the adrenal (Levi-Montalcini, 1987). We and others have established that NGF also alters immune function, in vitro and in vivo (Thorpe et al, 1988), following our original observation that there is specific and saturable binding of NGF to cell surface receptors on 20%-40% of rat lymphocytes (Thorpe et al, 1987).

It was the original goal of this project: 1. To characterize the NGF receptor on lymphoid cells and 2. to determine the effects of NGF on lymphocytes with an aim to understanding the physiological and developmental role of NGF in the immune system. Our approaches, as outlined, were to characterize NGF binding in different rodent and human lymphoid tissues and to screen possible NGFR bearing cell lines using equilibrium binding of ^{125}I-NGF to solubilized receptor preparations and also to crosslink such ^{125}I-NGF to the NGF receptor (NGFR) and carry out SDS-PAGE analysis of immunoprecipitated ^{125}I-NGF crosslinked to its receptor. We also embarked on a project to develop RP-HPLC techniques for the study of NGFR on lymphoid cells. Lastly, we assessed NGF action on lymphoid cells by measuring the effect of NGF on lymphocyte incorporation of ^{3}H-Thd into acid precipitable material as an index of DNA synthesis and cellular proliferation.

Progress Report.

During the past year our major accomplishments have been: To demonstrate that most unstimulated rodent thymocytes display NGF binding properties, NGFR protein and NGFR mRNA. We have found that the NGFR m/RNA in thymocytes is 4.1 Kb or 0.4 Kb larger than that found in neurons. The work on thymocytes has been submitted to the Journal of Immunology (see attached manuscript, Thorpe et al 1989). The finding of a 4.1 Kb NGFR mRNA was obtained with Northern analysis using an NGFR cDNA probe obtained from Eric Shooter at Stanford. We have applied RP-HPLC to the characterization of the NGFR present on lymphocytes. Here we will outline the progress of the effect of NGF on DNA synthesis and RP-HPLC analysis of the NGFR. Thymocytes were isolated, rapidly frozen and stored at -70 degrees C until time of RNA isolation. Total RNA was extracted by a modification of the guanidinium isothiocyanate (G-SCN) method (Maniatis et al. 1982). Poly (A+) RNA was isolated by a single passage of 700ug of total thymocyte RNA through an oligo (dt) column (Avin and Leder, 1972). Samples were loaded onto a gel of 1.2% agarose/8% formaldehyde in 1X MOPs running buffer. LaneA: 10ug of thymocyte poly(A+) RNA; Lane B: 40ug of thymocyte poly(A-) RNA. Samples were run at 25 volts (1.8V/cm gel) for 14 hours. The samples were then transferred to nitrocellulose (.45um pore size) by standard blotting technique in 20X SSC. The nitrocellulose was baked at 80 degrees C for two hours in a vacuum oven. After baking the membrane was placed in a plastic heat sealed bag that contained 10ml of prehybridization buffer (50% formamide/5x SSPE/5X Denhart's/1mM ATP/0.1% SDS/200ug denatured salmon sperm DAN/ml buffer) and prehybridized at 42 degrees C for 5 hours. The buffer and hybridization conditions are essentially the same as those found in Radeke et al, 1987. Following prehybridization, the buffer was replaced with an identical buffer that contained 10% dextran sulphate and 3 x 10^6 cpm/ml of a random primed ^{32}P-labeled cDNA for the NGFR (specific activity of probe, 3 x 10^8 dpm/ug DNA). The labeled cDNA was generated by using a random labeling kit (BIO-RAD catalog no. 170-3557) and 30ng of a T7 plasmid with an insert containing the cNGFR (kindly provided by Dr. Eric Shooter). The membrane was hybridized for 48 hours at 42 degrees C and then washed four times for 30 minutes in 0.2X SSC/0.1% SDS. After washing the membrane was air dried and subjected to overnight autoradiography in a lightproof cartridge with Kodak X-OMAT film and a Dupont Cronex intensifying screen.

The labeled cDNA hybridized to a single species of RNA of approximately 4.1 kb (as determined by 18S and 28S RNA measurements). It did not hybridize to anything in either the poly(A-) sample or in controls with rat liver poly(A+) RNA (not shown). This 4.1 kb band is somewhat larger than expected since previous studies have shown the NGFR message to be approximately 3.7 kb in the rat. It is unlikely that the size discrepancy is artefactual since we have used this procedure to label a mRNA of approximately 3.7 kb in both PC12 cells and rat basal forebrain (unpublished observations).

Binding of NGF to cell surface receptors (NGFR) is the first obligatory step to NGF action. Although the amino acid sequence for NGFR is known, the identification of a single gene for NGFR does not explain the existence of two forms of NGF binding activities and of the several reported molecular weights of NGFR. We report here on the partial isolation of several molecular species of NGFR from PC12 cells using lectin chromatography, reverse-phase HPLC and NGFR-specific monoclonal antibody 192 (mAb 192 to rodent NGFR) immuno-precipitation. In general we found a tendency for the higher molecular-weight species of NGFR protein to be eluted in the more hydrophobic fractions. Since the 117 and 133 kDa species must contain a mAb 192-recognizable subunit, we would expect the 76 kDa mature form of NGFR to be a component there too. Thus, the different molecular species of NGFR detected by HPLC, immuno precipitation and SDS-PAGE may result from generation of truncated forms of NGFR, the presence or absence of sugar residues on the NGFR protein the association of NGFR with a receptor-associated protein. Since wheat germ agglutinin (WGA) or tunicamycin (TM) do cause shifts in the proportion of high affinity to low affinity receptor and acetyl-L-carnitine (ALC) upregulates levels of NGF binding in PC12 cells, we were not surprised to find that treatment of PC12 cells with ALC or WGA induces the appearance of molecular species of NGFR with molecular weights of about 93kDa and 155kDa and that WGA or TM treatment reduced the low molecular-weight species of NGFR. This is consistent with the hypothesis that the low affinity state of NGFR resides in a low molecular-weight species and that the high affinity state of NGFR resides in a high molecular-weight species.

The human neuroblastoma cell line SY5Y was also used in this study of NGF and NGFR. NGF treatment of SY5Y induces differentiation events that are similar to the effect of NGF on rat pheochromocytoma PC12 cells. We found that the predominant presence of the high molecular weight species of NGFR in neuroblastoma SY5Y cells is consistent with the predominant presence of the high affinity NGF binding activity reported for this cell line. The expression of a 93 kDa species of NGFR in neuroblastoma cells may be due to an over expression of a 16 kDA receptor-associated protein. In addition, we demonstrated that the expression of different species of NGFR could be modified by treatment with retinoic acid (RA).

At that point we did a comparative study of the species of NGFR present in astrocytes and splenocytes since NGF has been reported to have a effects on cellular proliferation for both. We also found some time ago that the NGF binding activity displayed by astrocytes was very similar to that reported by us on lymphocytes. The unique pattern of NGFR displayed by lymphocytes may be related to the mitogenic effects of NGF there. Our results would suggest that the NGFR pattern in lymphocytes may result from an excess in expression of receptor-associated proteins as compared to NGFR.

Since only a small proportion of lymphocytes were NGFR bearing and/or NGF responsive we undertook to determine if NGFR were present on undifferentiated rat thymocytes and to assess possible in vitro biological actions of NGFR on proliferative response of these cells. To explore the functional modulation of NGF on thymocyte activation, unfractionated cells were cultured in the presence of varying concentrations of NGF added at the initiation of culture. NGF showed a significant time and dose dependent stimulation of proliferation with maximal ^{3}HThd incorporation at 96 hours of culture. The degree of responsiveness of the cells to NGF stimulation varied from experiment to experiment. In some studies a maximally increased proliferative response was seen at 0.1 ug/ml with a plateauing of the response at the higher dosages of NGF while in others a classical dose dependent increased thymidine uptake was observed.

The ability of NGF to modulate thymocyte proliferation in the presence of mitogens was determined by coculturing cells with NGF and ConA. After 72-96 hours costimulation with NGF and ConA a significant synergistic enhancement of ConA induced ^{3}HThd uptake was observed. This response to NGF by ConA stimulated cells was only found at lower concentrations (0.1-0.5 ug/ml) of ConA.

The ability of NGF to synergize with lymphokines was assessed by coculturing the ligand with IL2. The addition of both NGF and IL2 at the initiation of cultures, potentiated the mitogenic signal of IL2 in a concentration dependent manner. Proliferation in response to IL2 and NGF was highest in cultures with serum containing media (CM), although significant enhancement of IL2-driven mitogenesis by NGF was also present in SFM.

Our in vitro studies indicate that NGF is functionally active and can modulate in a concentration dependent manner the mitogenic response of cultured thymocytes. The enhancement of proliferation by NGF occurs alone and in concert with other mitogenic substances in a concentration dependent manner. This response occurs in the presence or absence of serum, supporting a specific and direct effect of NGF on the enhancement of thymocyte activation. The specificity of the response is also supported by the inhibitory effects of antisera against NGF on the proliferative response of NGF stimulated cells.

The in vitro reactivity of thymocytes to NGF stimulation is comparable with those seen in NGF culture studies of rat splenic cell populations (Thorpe and Perez-Polo, 1987). Although the maximal response of these cells occurs at levels significantly above those considered physiological it is unknown what concentrations of NGF can be induced within the local microenvironment during an immunoinflammatory response. The lowest concentration of NGF which enhances the proliferative activities of both thymocytes and splenocytes correspond to that of the receptor saturation concentration. In addition, preliminary findings suggest that NGF effects on lymphokine production by cultured lymphocytes is seen at NGF concentrations below those

needed for receptor saturation (Thorpe et al, unpublished).

Presently the mechanisms by which NGF potentiates cell cycle events are unknown, however they may include its interactions within the IL2/IL2R pathway. This hypothesis is supported by several observations including NGF upregulation of IL2R expression on human peripheral lymphocytes (Thorpe et al, 1987), and more recently preliminary results demonstrating NGF potentiation of IL2 synthesis by cultured lymphoid cells (Thorpe et al, 1989). The effects of NGF on IL2R expression and IL2 synthesis by cells of the thymus are unknown, however the presence of IL2R on functionally mature populations of thymocytes and their response to varying concentrations of IL2 would tend to support the possibility of its activity on the modulation of the IL2/IL2R cascade.

Mounting evidence continues to accrue that suggests a role for NGF in the maintenance of immune homeostasis. (reviewed in Thorpe et al, 1988b), and in the neuroimmune axis as a bidirectionally acting mediator in the communication network between the neuroendocrine and immune system. Our present results linked with other reports on NGF interactions during thymic development, indicate that an important direction for future studies on NGF interaction within the thymus will be to define its role in the thymic embryonic development and in T-cell ontogeny.

One project that did <u>not</u> succeed was our search for established cell lines of lymphoid origin that would express NGFR. Given our success with thymocytes, we abandoned that project half way through the year.

References

Whittemore et al 1989 Radecke et al 1987 (Avin and Leder, 1972) (Maniatis et al. 1982)Thorpe et al, 1987; Thorpe, et al, 1987; Thorpe and Perez-Polo, 1987; Thorpe et al, 1988; Thorpe et al, 1989; Morgan et al, 1989).

(Levi-Montalcini, 1987 Hulsebosch, CE, J.R. Perez-Polo and R.E. Coggeshall. In vivo and anti-NGF induces sprouting of sensory axons in dorsal roots. J. of Hulsebosch, CE, J.R. Perez-Polo and R.E. Coggeshall. In vivo and anti-NGF induces sprouting of sensory axons in dorsal roots. J. of Comparative Neurology 259:93 445-451, 1987.

Hulsebosch, CE, R.E. Coggeshall and J.R. Perez-Polo. Persistence of anti-NGF induced dorsal root axons: possible penetration into the mammalian spinal cord. Brain Res., 411:267-274, 1987.

Thorpe, L.W., K. Werrbach-Perez and J.R. Perez-Polo. Effects of nerve growth factor on the expression of interluekin 2 receptors on cultured human lymphocytes. Ann. N.Y. Acad. Sci. 496:310-311,

1987.

Thorpe, LW, Foreman, J., Taglialatela, G., Perez-Polo JR. Nerve growth factor interactions in the immune system. Characterization of specific NGF receptors on rat thymocytes and the augmentation of thymocyte proliferation in vitro. Journal of Neuroimmunology. Submitted 8/18/89.

Shan, D.E., Werrbach-Perez, K., and Perez-Polo, J.R. HPLC study of the nerve growth factor receptor in human neuroblastoma cells and modifications of the receptor with retinoic acid. Brain Research. Submitted 8/22/89

Shan, D.E., Werrbach-Perez, K. and Perez-Polo, J.R. Effect of acetyl-L-carnitine, wheat germ agglutinin and tunicamycin on nerve growth factor receptor molecular species in PC12 cells. Submitted J. of Neurochemistry. Submitted 7/03/89.

Shan, DE, Beck, CE and Perez-Polo, JR. Isolation of five molecular species suggestive of nerve growth factor receptor from PC12 cells using reverse-phase high performance liquid chromatography. Submitted J. of Neurochemistry. Submitted 7/03/89.

Shan, DE, and Perez-Polo, JR. HPLC Study of the effects of nerve growth factor and high potassium on the expression of nerve growth factor receptor in PC12 cells. Submitted J. of Neuroscience. Submitted 8/18/89.

Shan, DE, Thorpe, LW and Perez-Polo. Comparison of nerve growth factor receptor partially purified by reverse-phase HPLC from astrocytes and lymphocytes. J. Neurochemistry. Submitted 7/03/89.

A LIST OF ANY POTENTIALLY PATENTABLE INVENTIONS OR PATENT DISCLOSURES FILED. (None)

LIST OF PAPERS PUBLISHED DURING CONTRACT PERIOD

Haskell, BE, RW Stach, K Werrbach-Perez and J. Regino Perez-Polo. Effect of retinoic acid on nerve growth factor receptors. Cell Tissue Res. 247:67-73, 1987.

Thorpe, Larry W., Robert W. Stach, George Hashim, Dario Marchetti, and J. Regino Perez-Polo. Receptors for Nerve growth factor on rat spleen mononuclear cells. J. of Neuroscience Res. 17:128-134, 1987.

Stach, R.W. and J.R. Perez-Polo. Binding of nerve growth factor to its receptor. J. of Neuroscience Res. 17:1-10, 1987.

Hulsebosch, CE, J.R. Perez-Polo and R.E. Coggeshall. In vivo and anti-NGF induces sprouting of sensory axons in dorsal roots. J. of Comparative Neurology 259:93 445-451, 1987.

Marchetti, Dario and J. Regino Perez-Polo. Nerve growth factor receptors in human neuroblastoma cells. J. of Neurochemistry 49:475-486, 1987.

Hulsebosch, CE, R.E. Coggeshall and J.R. Perez-Polo. Persistence of anti-NGF induced dorsal root axons: possible penetration into the mammalian spinal cord. Brain Res., 411:267-274, 1987.

Thorpe, L.W., K. Werrbach-Perez and J.R. Perez-Polo. Effects of nerve growth factor on the expression of interluekin 2 receptors on cultured human lymphocytes. Ann. N.Y. Acad. Sci. 496:310-311, 1987.

Marchetti, Dario, R.W. Stach, R. Saneto, J. de Vellis and J.R. Perez-Polo. Binding constants of soluble NGF-receptors in rat oligodendrocytes and astrocytes in culture. Biochem. & Biophys. Comm. 147 1:422-427, 1987.

Stach, RW, CR Lyons and J.R. Perez-Polo. Characteristics of partially purified nerve growth factor receptor. J. of Neurochemistry 494:1280-1285, 1987.

Perez-Polo, J. Regino and Karin Werrbach-Perez. De novo synthesis of NGF subunits in S-180 mouse sarcoma cell line. Neurochemical Research 12:875-883, 1987.

Bostwick, JR, SH Appel and J. Regino Perez-Polo. Distinct influences of nerve growth factor and a central cholinergic trophic factor on medial septal explants. Science 422:92-98, 1987.

Thorpe, Larry W and J. Regino Perez-Polo. The influence of nerve growth factor on the in vitro proliferative response of rat spleen lymphocytes. J. of Neuroscience Res. 18:134-139, 1987.

Khan, Talat, Barth Green and J.R. Perez-Polo. Effect of injury on nerve growth factor uptake by sensory ganglia. J. of Neurosci. Res. 18:562-567, 1987.

Wright, LL, C. Beck and JR Perez-Polo. Sex differences in nerve growth factor levels in superior cervical ganglia and pineals. Int. J. Devl. Neuroscience 5:5/6, 383-390, 1987.

Wright, LL, D. Marchetti and JR Perez-Polo. Effects of gonadal steroids on nerve growth factor in sympathetic and sensory ganglia of neonatal rats. Int. J. Devl. Neuroscience 6:3 217-222, 1988.

Angelucci, L., MT Ramacci, G. Taglialatela, C. Hulsebosch, B. Morgan, K. Werrbach-Perez and R. Perez-Polo. Nerve growth factor binding in aged rat CNS: Effect of acetyl-L-carnitine. J. Neurosci. Res. 20:491-496, 1988.

Reynolds, C Patrick and J. Regino Perez-Polo. Nerve growth factor induced neurite outgrowth in clone derived from NGF insensitive

human neuroblastoma cell line. Int. J. Devl. Neurosci. 7(2):125-132, 1989.

Morgan, B., D. Marchetti, LW Thorpe and Perez-Polo, JR. Expression of nerve growth factor receptors by human peripheral blood mononuclear cells. J. of Neuroscience Res. 23:41-45, 1989.

Vanella, A, R Avola, DF Condorelli, A Campisi, A Costa, AM Giuffrida Stella and JR Perez-Polo. Antioxidant enzymatic activities and resistance to oxidative stress in primary and subcultured rat astroglial cells. Int. J. Devel. Neurosci. 7:233-241, 1989.

Perez-Polo, J. Regino and Karin Werrbach-Perez. In vitro model of neuronal aging & development in the nervous system: In: Model Systems of Development & Aging of the Nervous System, Vernadakis A., (ed.), Martinus Nujhoff, Boston, pp. 433-442, 1987.

Perez-Polo, J. Regino. Introduction. In: Neuronal Factors Ed. J.R. Perez-Polo, CRC Press, Boca Raton, Fl. pp. 1-7, 1987.

Perez-Polo, J. Regino. Bioassays. In: Neuronal Factors. (Ed) JR Perez-Polo, CRC Press; Boca Raton, Fl. pp. 9-51, 1987.

Perez-Polo, J. Regino. Purification of PNS factors. In: Neuronal Factors, (ed) JR Perez-Polo, CRC Press; Boca Raton, Fl. pp. 117-149, 1987.

Perez-Polo, J. Regino and K. Werrbach-Perez. Role of nerve growth factor in neuronal injury and survival. In: Cellular and Molecular Aspects of neural development and regeneration (eds) A. Gorio, JR Perez-Polo, J. de Vellis, B. Haber, Springer Verlag, Heidelberg, pp399-410, 1988.

Thorpe, LW, B. Morgan, C. Beck, K. Werrbach-Perez and JR Perez-Polo. Nerve growth factor and the immune system. In: Cellular and Molecular Aspects of Neural Development and Regeneration (eds) A. Gorio, JR Perez-Polo, J deVellis and B Haber, Springer Verlag, Heidelberg pp. 583-594, 1988.

Perez-Polo, JR, K. Werrbach-Perez, MT Ramacci, G. Taglialatela, B. Morgan, L. Angelucci. Role of nerve growth factors in neurological disease. In: Current Problems in Senile Dementias Number 2 (eds) A. Agnoli, J. Cahn, N. Lassen, R. Mayeux, John Libbey Eurotext, London pp. 15-25, 1988.

Thorpe, Larry W; Robert W. Stach, Brent Morgan and J. Regino Perez-Polo. The biology of nerve growth factor: its interaction with cells of the immune system. In: Neural Control of Reproductive Function. (eds) J. Lakoski, J. Regino Perez-Polo and D. Rassin; Alan Liss, Inc., New York, pp 351-369, 1989.

Shen, Rong-sen; Yixian Zhang and J. Regino Perez-Polo. Regulation of GTP Cyclohydrolase I and dihydropteridine reductase in rat pheochromocytoma PC12 cells. J. Enzyme Inhibition, 3 119-126, 1989.

IN PRESS:

Perez-Polo, J. Regino, Werrbach-Perez K, Jackson, G, Beck C, Marchetti D, Morgan B, Taglialatela G, Ramacci MT and Angelucci L. Neuronotrophic Factors in Aged Rodent Central Nervous System. In: Aging Brain & Dementia: New Trends in Diagnosis and Therapy (ed) Battistin, L. Alan Liss.

Perez-Polo JR, Ramacci MT, Taglialatela G, Werrbach-Perez K, Morgan B, Marchetti D and Angelucci L. Possible roles of growth factors in the treatment of neurological diseases.

Perez-Polo, J. Regino. Role of trophic factors in neuronal aging. In: Molecular Aspects of Development and Aging of the Nervous System. (eds) Lauder JM, Privat A, Giacobini E, Timiras PS & Vernadakis A.

Perez-Polo, J. Regino, Karin Werrbach-Perez, Dario Marchetti, Brent Morgan, Giulio Taglialatela, Maria Teresa Ramacci and Luciano Angelucci. NGF in pathologies correlated to cerebral aging. In: Brain metabolism and aging. (eds) A. Bertelli.

Perez-Polo, J. Regino. The Nerve Growth Factor Receptor. In: Trophic Factors and the Nervous System. (ed) Neff, NH, Raven Press.

Werrbach-Perez, Karin, George Jackson, Dario Marchetti, Brent Morgan, Larry Thorpe, & J. Regino Perez-Polo. Growth Factor-Mediated protection in aging CNS. (Eds)CH Phelps.

Jackson, George R., Lori Apffel, Karin Werrbach-Perez and Regino Perez-Polo. Role of Nerve Growth Factor in oxidant -antioxidant balance and neuronal injury I. Stimulation of hydrogen peroxide resistance. J. of Neurosci. Res.

Jackson, George R., Karin Werrbach-Perez, and J. Regino Perez-Polo. Role of nerve growth factor in oxidant-antioxidant balance and neuronal injury II. A conditioning lesion paradigm. J. of Neurosci. Res.

Jackson, George R., Brent Morgan, Karin Werrbach-Perez and J. Regino Perez-Polo. Model for the study of conditioning lesions in the pathophysiology of the nervous system. In: International Society for Myochemistry (ed). G. Benzi

Werrbach-Perez and Regino Perez-Polo. Nerve growth factor action in the central nervous system: A cell marker for change or an intrinsic agent of change? (ed) AM Giuffrida-Stella, J. deVellis J.R. Perez-Polo, Alan Liss, New York.

Angelucci, L., Imperato, A., Ramacci, M.T., Taglialatela, G. and Perez-Polo, J.R. Neurotransmission and neurotrophism in the aging brain. (eds). AM Guiffrida-Stella, J. deVellis & J.R. Perez-Polo, Alan Liss, New York.

Angelucci, L., Amenta, F., Conti, F., Miccheli, A., Ramacci M.T., Taglialatela, G. Teng, J.I., Werrbach-Perez, K. and Perez-Polo, J.R. Acetyl-L-carnitine and receptors in the aging rat's hippocampus. (ed) CH Phelps.

Taglialatela, G., Angelucci, L., Ramacci, MT, Foreman, PJ and Perez-Polo, JR. 125-Beta-Nerve growth factor binding is reduced in rat brain after stress exposure. J. Neuroscience Res. 25:1 January, 1990.

Thorpe, LW, Jerrells, TR and Perez-Polo, JR. Mechanisms of lymphocyte activation by nerve growth factor.

SUBMITTED

Thorpe, LW, Foreman, J., Taglialatela, G., Perez-Polo JR. Nerve growth factor interactions in the immune system. Characterization of specific NGF receptors on rat thymocytes and the augmentation of thymocyte proliferation in vitro. Journal of Neuroimmunology. Submitted 8/18/89.

Shan, D.E., Werrbach-Perez, K., and Perez-Polo, J.R. HPLC study of the nerve growth factor receptor in human neuroblastoma cells and modifications of the receptor with retinoic acid. Brain Research. Submitted 8/22/89

Shan, D.E., Werrbach-Perez, K. and Perez-Polo, J.R. Effect of acetyl-L-carnitine, wheat germ agglutinin and tunicamycin on nerve growth factor receptor molecular species in PC12 cells. Submitted J. of Neurochemistry. Submitted 7/03/89.

Shan, DE, Beck, CE and Perez-Polo, JR. Isolation of five molecular species suggestive of nerve growth factor receptor from PC12 cells using reverse-phase high performance liquid chromatography. Submitted J. of Neurochemistry. Submitted 7/03/89.

Shan, DE, and Perez-Polo, JR. HPLC Study of the effects of nerve growth factor and high potassium on the expression of nerve growth factor receptor in PC12 cells. Submitted J. of Neuroscience. Submitted 8/18/89.

Shan, DE, Thorpe, LW and Perez-Polo. Comparison of nerve

growth factor receptor partially purified by reverse-phase HPLC from astrocytes and lymphocytes. J. Neurochemistry. Submitted 7/03/89.