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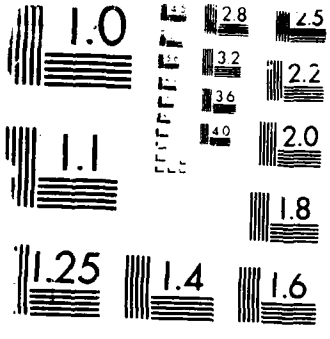

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The nerve growth factor protein, NGF, has been shown to play a physiologic role in the development and regeneration of the peripheral nervous system, acting on sensory and sympathetic ganglia. In the central nervous system, NGF induces choline acetyltransferase 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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ANNUAL REPORT

Nerve Growth Factor Effects on the Immune System
ONR Contract No. N00014-87-K-0364

During the last 7 months of this contract period we have focused our efforts on the characterization of the response of rodent splenic lymphocytes to NGF. Also, we have investigated the particular rodent lymphocyte population that binds to NGF. Lastly, we are developing techniques for the quantitation of NGF receptors, NGFR.

Visualization of binding of NGF to rat splenic mononuclear (MC) and human peripheral blood mononuclear cells (PBMC) was detected by immunofluorescence using NGF with heterologous anti-NGF as well as NGFR specific monoclonal antibodies. NGFR positive cells comprised approximately 25-50 percent of the mononuclear cells and peripheral blood mononuclear cells. To determine if NGFR positive cells were expressed on selected lymphocyte populations, rat MC were doubly stained for NGFR using IgG-192 (Chandler et al, 1984) and monoclonals (McMasters & Williams, 1979; Brideau et al, 1980; Sternberg et al, 1986; White et al, 1986) which distinguish T cells (OX 19), T cell subsets (W3/25, OX 8), and Ia positive (OX 4) B cells. These studies revealed that NGFR were found on a variable percentage of both the T cell and B cell population (Thorpe et al, unpublished). In addition, it appeared that within the T lymphocytes, the majority of the NGFR positive cells were phenotypically T suppressor/cytotoxic cells (ON 8); while only a small percentage of T helper cells (W3/25) were NGFR positive. Although these results are of a preliminary nature, they suggest that NGFR are selectively expressed on a yet undefined population of T and B lymphocytes. This is not unique to NGF. Beta-adrenergic and substance P receptors are selectively distributed among specific lymphocyte cell population subsets (Landmann et al, 1984; Payan et al, 1985). In addition, Simon, et al, (1986) have reported that stimulation of human lymphocytes in culture with angiotensin II selectively stimulates DNA synthesis in OKT-8 positive cells, the human T-suppressor/cytotoxic cell. Thus, NGF positive immunocytes may be a functionally distinct population that is reactive to the modulatory effects of NGF.

NGF binding studies of unfractionated MC and PBMC revealed a relatively rapid, saturable and reversible binding of NGF to solubilized membranes (Thorpe et al, 1987; Marchetti et al, in preparation). Scatchard analysis of binding data for rat MC NGFR showed an equilibrium dissociation constant of 10^{-9} M, consistent with the existence of a single low affinity binding site (Thorpe et al, 1987). Scatchard analysis of human PBMC gave a biphasic binding curve with Kd's of 10^{-11} and 10^{-9} M, consistent with the existence of both high and low affinity receptors on human cells (Marchetti et al, in preparation). Molecular weights of the NGFR on MC were determined by SDS-PAGE analysis of IgG-192 immunoprecipitated NGFR. Two molecular species of approximately 190 and 125 kilodaltons (KDa) were found (Thorpe et al, 1987). Molecular weight determination of solubilized PBMC NGFR followed by lentil-lectin chromatography and

SDS-PAGE, revealed two proteins with molecular weights of 133 and 100 KDa.

In vitro culture studies have demonstrated that lymphocytes and/or accessory cells are targets of NGF, presumably through receptor-mediated mechanisms. The addition of NGF to cultures of rat MC resulted in the stimulation of blastogenic activity both in the presence and absence of T and B cell mitogens (Thorpe & Perez-Polo, In Press). In the absence of mitogen NGF stimulated DNA synthesis in MC as measured by tritiated thymidine uptake after 96 hours in culture. Significant increases in thymidine incorporation in a dose dependent fashion were noted at NGF concentrations of 0.1-10 ug/ml, corresponding to molar concentrations of 3.7-370 nM. The minimal effective concentration of NGF was 3.7 nM, similar to the level of NGF required for occupancy of half the binding sites on unfractionated rat splenic MC. The highest level of stimulation was observed at 10 ug/ml. In addition, the response of splenic MC to mitogenic stimulation was also significantly augmented in the presence of NGF. In the presence of mitogen, the lymphoproliferative response was dependent on the concentration of the mitogen and the concentration of NGF. For the T cell mitogen concanavalin A (Con A), the highest levels of NGF stimulation of DNA synthesis were observed at suboptimal concentrations of the mitgoen (Fig 1).

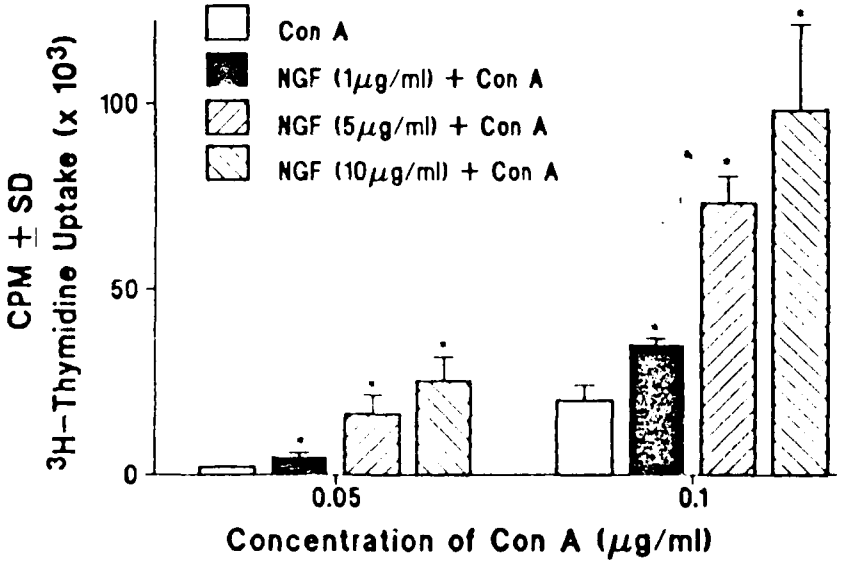

Figure 1 Enhancement of the response of rat spleen MC to NGF at suboptimal concentrations of the T cell mitogen Con A. Augmentation by NGF was dose-dependent and significantly higher than mitogen alone at all concentrations.

Similar findings of a dose dependent NGF augmentation were observed for another T-cell mitogen, phytohemagglutinin. Additionally, there was potentiation by NGF of the B-cell proliferative response in the presence of the mitogen lipopolysaccaride (LPS) in a dose dependent way. Unlike the T-cell mitogen studies, where the optimal responses were seen at suboptimal mitogen concentrations, NGF potentiation of LPS stimulated cells took place over a broad range of mitogen

concentrations. In experiments designed to examine the effects of serum on the NGF induced proliferative response a serum free media, Ventrex HU-1, was used. When splenic cells were cultured in Ventrex and ConA, NGF augmented the ConA response. Here the response to NGF was again significant at suboptimal concentrations of mitogen whereas the optimal NGF effect took place at lower NGF concentrations, in the 0.1-1 ug/ml range, in sharp contrast to the previous results of maximal stimulation at 10 ug/ml of NGF obtained in the presence of serum. Previous studies have shown that NGF binds to serum components (Perez-Polo et al, 1980). Therefore, the high concentration of NGF needed for maximal NGF effects on rat splenic cell proliferation in serum containing media might be due to interference by serum factors.

Mitogens are polyclonal activators of immune cells. A better in vitro correlate of the in vivo immune response to antigentic challenge is that of the mixed lymphocyte response (MLR). The study by Manning and her colleagues (1985) found no increased MLR activity by NGF in rat mixed mouse MLC, an increased stimulation by NGF was noted in cultures with 10 ug/ml NGF. Preliminary results of rat mixed lymphocyte cultures stimulated with NGF have demonstrated that NGF also potentiates rat MLC reactivity and that this augmentation by NGF was only seen at concentrations of 10 ug/ml NGF (Fig. 2). In contrast to the mitogen studies, here NGF at concentrations of 1 ug/ml, or less, had no effect on the MLR. The NGF potentiation of the proliferative response is not restricted to differentiated lymphocytes. Thymocytes from young rats also responded in culture to NGF in the presence of ConA (Fig. 3). However, there was no effect on thymidine uptake by thymocytes cultured with NGF in the absence of mitogen (Thorpe, unpublished).

The mechanisms of action of NGF effects on the proliferation of MC and the mitogen stimulated lymphocytes and thymocytes is not known. Lymphocyte mitogenesis is the result of a complex series of interactions involving direct lymphocyte and macrophage cell mediated interactions and their response to monokines and lymphokines (Cantrell & Smith, 1984).

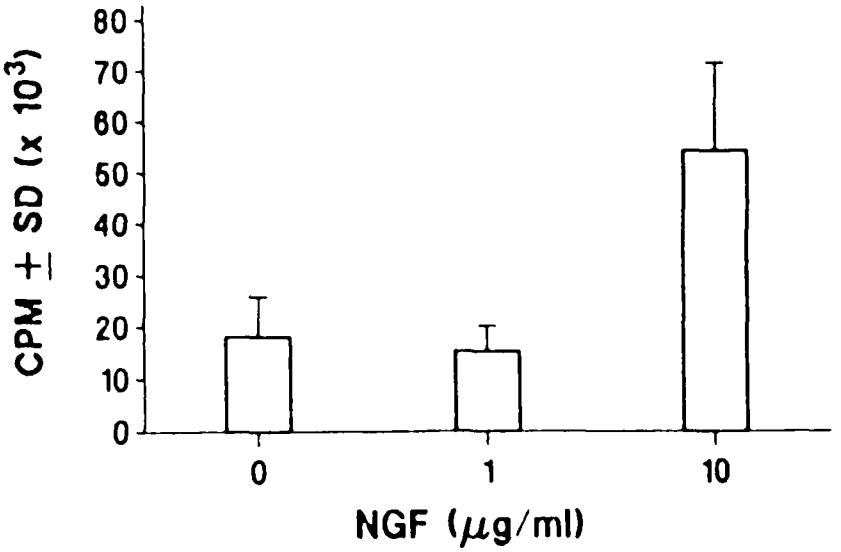

Figure 2. Effect of NGF (10 ug/ml) on the MLR response of rat one-way mixed lymphocyte cultures (Lewis vs irradiated BN).

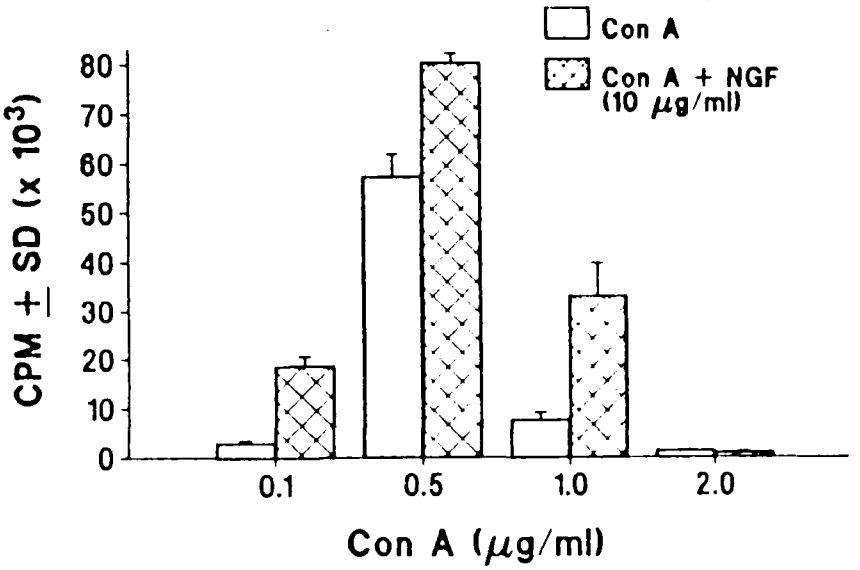

Figure 3. NGF enhancement of proliferation of thymocytes in the presence of Con A. NGF (10 ug/ml) significantly potentiated the response to mitogen through a defined range of Con A concentrations.

Since lymphoproliferation occurs in response to a number of different cellular interactions, the role of the different NGFR positive cell types (lymphocytes and macrophages) in the NGF induced blastogenic response is not obvious. An early event obligatory to the activation of the cell cycle of lymphocytes is the stimulation of interleukin 2 (IL-2) synthesis and the upregulation of IL-2 receptors (IL2-R). In the absence of antigen or mitogen stimulation, NGF increased the expression of IL2-R on cultured human PBMC (Thorpe et al, in press). The upregulation of receptors was observed after approximately 72 hours in culture and preceded the increase in DNA synthesis observed after 96 hours of culture. Similar IL2-R modulation by thymic hormones has been reported by Sztein et al (1986). The cellular events associated with the IL2-R induction by NGF are unknown.

We have determined solvation procedures for immunoprecipitates of NGFR that are compatible with HPLC reverse phase chromatography. Using 0.1% TFA and 0.1% Formic acid we can recover 70% of NGFR. We plan to use a C4 ultrapore column as an analytical tool to compare NGFR protein from immunogenic tissues to its neuronal counterpart.

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