Journal of the Neurological Sciences 333 (2013) 30–36

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Direct effects of secretory products of immune cells on neurons and glia

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ARTICLE INFO

Article history:
Received 31 March 2013
Received in revised form 30 May 2013
Accepted 2 June 2013
Available online 2 July 2013

Keywords:
B cells
Cytokines
FAT10
Glia
IL-6 signal transducer
Multiple sclerosis
Neuroimmunology
Neurons

ABSTRACT

This review summarizes recent research contributions by Dr. Robert Lisak in collaboration with Dr. Joyce Benjamins on direct effects of secretory products of immune cells on neurons and glia. Highlights from studies analyzing cytokine-induced changes in early gene expression in mixed CNS glial cultures focus on comparison of potential damaging effects of pro-inflammatory cytokines versus protective effects of downregulatory cytokines. The three categories of changes examined include (a) immune-related molecules, (b) neurotrophins, growth factors and structural proteins, and (c) molecules associated with metabolism, signaling and regulation. Subsequent studies in CNS neuronal cultures showed that early responses of neurons to cytokines were fewer in number and lower in magnitude than in glia, consistent with the idea that microglia and astroglia serve as "first responders" to inflammatory signals. To explore the hypothesis that B cells of patients with multiple sclerosis secrete soluble products damaging to oligodendroglia (OL), in collaboration with Dr. Bar-Or at Montreal Neurologic Institute, we compared secretory products of cultured B cells from relapsing remitting multiple sclerosis (RRMS) patients and healthy controls. In support of the hypothesis, 7 supernatants from RRMS B cells induced death of rat OL in vitro, while 3 of 4 control samples did not.

Contents

1. Tribute to Robert Lisak, M.D.

This review summarizes recent collaborative efforts with Bob Lisak. In addition to our many scientific discussions and collaborations over the years, I have been privileged to work with Bob in growing and developing the Department of Neurology at Wayne State University. Bob's extensive knowledge of neuroimmunology and my interest in glial biology lead to investigations on the effects of cytokines on gene expression in glia, and effects of products secreted by B cells from MS patients on glia.

Bob's expertise in neuroimmunology originated during his fellowship years at the NIH in the laboratory of Dr. Marian Kies, and their studies on experimental allergic encephalomyelitis induced with myelin basic protein [1–4] and then as a fellow with Dr. Burt Zweiman at the University of Pennsylvania [5,6]. Early in Bob's career, a sabbatical with Martin Raff resulted in advances in identifying phenotypic markers for neurons and glia [7,8]. He continues his many collaborations with colleagues at Wayne State University, around the country and abroad.

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2. Introduction

In multiple sclerosis, inflammatory cells are found in both active and chronic lesions, and it is increasingly clear that cytokines are

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http://dx.doi.org/10.1016/j.jns.2013.06.001

involved directly and indirectly in both formation and inhibition of lesions. A number of studies have investigated molecular changes in lesions of MS patients by analyzing MRS signals [9] or examining post-mortem tissue by immunocytochemistry, proteomics or genomics [10–18]. While these studies have been informative, these approaches are not able to address the roles that cytokines play in the initiation of early changes in NAWM and NAGM that could subsequently lead to acute WM damage and chronic neurodegeneration in MS. We have used primary CNS cell cultures to identify the initial early effects of secretory products of immune cells on glia and neurons. The long-term goal of our studies is to identify molecules that initiate or inhibit lesion formation as potential targets for prevention or protection in MS and other neurodegenerative diseases.

3. Cytokine-induced changes in early gene expression in CNS glia in vitro

In a series of studies, we showed that cytokine mixtures typical of Th1 or Th2 lymphocytes or monocyte/macrophages (M/M1 monocytes) each induce unique molecular changes in glial cells [19–21]. To examine changes in gene expression, we used gene array analysis to assess the early effects of the three different cytokine mixtures (Table 1) on mixed CNS glia in culture. We compared the effects of cytokines typical of Th1 and Th2 lymphocytes and monocyte/macrophages (M/M) on CNS glia in vitro after 6 h of treatment. We used mixed glial cultures from rat brain (Fig. 1) as a model of the glial environment in vivo; these cultures contained 40% oligodendroglia, 40% astroglia, 10% microglia, and 10% oligodendroglial progenitors and unidentified cells. In our initial gene array studies, we treated the cultures for 6 h with the cytokines to examine changes in immediate early gene expression at a time when secondary effects from downstream changes in gene and protein expression were minimal.

3.1. Changes in glial gene expression for immune-related molecules

Our first series of analyses compared the effects of the Th1, Th2 and M/M cytokines on expression of immune-related molecules in CNS glia [19]. Overall, the "pro-inflammatory" Th1 and M/M cytokines induced robust regulation at 6 h in expression of many immune-related molecules, while the "immunomodulatory" Th2 cytokines affected many fewer genes. Among the genes most markedly upregulated by both Th1 and M/M cytokines were VCAM-1, ICAM-1, IL-1α and IL-1β, as well as interferon-inducible factor 1. Of note, both M/M and Th2 cytokines upregulated osteoprotegerin, a decoy receptor for several members of the TNF receptor family, including RANK (receptor activator of NF-κB) and the receptor TRAIL. With regard to chemokines, Th1 and M/M cytokines upregulated expression of a number of genes, most robustly for CCL5 (RANTES) and CXCL10 (Mob-1). All three cytokines markedly downregulated IL-10, but upregulated IL-6. While no change was observed on the gene arrays for IL-6 signal transducing factor, all three cytokines downregulated a related protein, the leptin receptor. Given the pivotal role of IL-6st in receptor signaling for several cytokines, (IL-6, IL-11 and IL-27) and growth factors (CNTF, LIF), we investigated the effects of cytokines on its expression at longer time points [22]. Expression of both message and protein for IL-6st was decreased by all three cytokines between 1–5 days, and this decrease occurred in all three types of glia (Fig. 2). In the complement pathway, Th1 cytokines induced reciprocal regulation of the genes for CD55/DAF (decay accelerating factor), downregulated −6.8 fold, and C3/C5 convertase, upregulated 14 fold. CD55 binds to C3b and inhibits C3/C5 convertase [23]. Thus downregulation of CD55 and simultaneous upregulation of C3/C5 convertase, a key factor in triggering the complement membrane attack complex, could promote immunopathogenically mediated damage in the CNS.

Table 1
Components of cytokine mixtures.

Th1	IL-2, IFN-γ TNF-α, G-CSF
M/M	TNF-α. IL-1α, IL-1β, IL-6, IL-12p40
Th2	IL-4, IL-5, IL-10, G-CSF, TGF-β

All cytokines were recombinant proteins of rat origin except for G-CSF (mouse) and TGF-β (porcine). All individual cytokines were used at a final concentration of 10 ng/ml.

3.2. Changes in glial gene expression for neurotrophins, growth factors and structural proteins

We subsequently focused on cytokine-induced changes at 6 h in glial expression of genes with potential relevance for neuroprotection and axon/glial interactions, including neurotrophins and growth factors, their receptors and adhesion molecules. Each of the three cytokine mixtures induced a unique pattern of changes, with changes generally greater and potentially more harmful for the Th1 cytokine mixture than for M/M or Th2. Of note, BDNF was downregulated by the proinflammatory Th1 cytokines, but upregulated by the immunomodulatory Th2 cytokines (Fig. 3). Th1 cytokines downregulated the genes for trkb and p75, as well as NT3 and an alternative splice form of its receptor trkc. Using PCR, we confirmed these changes, and examined changes at 1 day and 3 days as well.

Other genes showing changes at the early 6 hour time point included cell adhesion molecules, connexins, deleted in colon cancer (DCC), and breast cancer gene BRCA1. DCC was downregulated by Th1 cytokines; after our original paper was published, DCC was shown to be critical, along with netrin, for the maintenance of axo-oligodendroglial paranodal junctions [24] and to regulate branching and expansion of OL membrane processes [25]. BRCA1, downregulated by all three cytokines, plays a role in activation of the sox2 gene which promotes conversion of OPC to neural stem cells [26]. We found downregulation of −5 fold of prolactin receptor by both Th1 and M/M cytokines, another change potentially relevant for OPC differentiation. Prolactin regulates OPC proliferation, so a reduction in prolactin receptor levels could inhibit the supply of OPCs available during remyelination [27,28].

The gene for BEST5 was upregulated 40 fold by Th1 cytokines and 5 fold by M/M cytokines. BEST5 is an interferon-inducible gene known to function in bone remodeling, but its expression in the CNS has not been previously reported, and its function in the CNS is not known. Uterine sensitization-associated gene protein 1 (USAG-1), a regulator of bone morphogenetic proteins (BMP) was downregulated by M/M cytokines; BMP play critical roles in OL, astrocye and neuron development [29,30]. Unexpectedly, no changes in expression for myelin-associated genes occurred at this early time point, suggesting that regulation of these genes may be secondary responses downstream of the initial cytokine-induced changes in gene and protein expression.

3.3. Changes in glial gene expression for molecules associated with metabolism, signaling and regulation

Many of the cytokine-induced changes we found in glial gene expression in culture [21] were similar to those seen in some types of MS tissues and in early inflammatory lesions in experimental autoimmune encephalomyelitis (EAE). In some cases, those changes were similar to those associated with neuroprotective mechanisms found in ischemic preconditioning following hypoxia. Normal appearing white matter (NAWM) and gray matter (NAGM) from MS brains showed many fewer changes in genes related to immune and stress responses than the more active MS lesions themselves [10–15,31]. Changes characteristic of ischemic preconditioning occurred in NAGM, where infiltration of immune cells is limited [16–18,32,33]. These changes included reduced expression of nuclear-encoded mitochondrial genes, genes related to ion homeostasis and neurotransmission and upregulation of genes and proteins associated with ciliary neurotrophic factor (CNTF) and signaling pathways [34]. Type III MS lesions, characterized by a primary oligodendrogliopathy with apoptotic