Invited Trends Article:

Retinoic Acid as a Regulator of Cytokine Signaling after Nerve Injury

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After an injury of the central nervous system it is of foremost clinical concern to prevent nerve cell degeneration and to develop strategies for the support of axonal regeneration. This requires an understanding of traumatic processes in the nervous system and their regulation by intercellular cytokine signaling. Although injury-induced temporal changes in gene expression of many cytokines have been described in this context, much less is known about their regulation. This review proposes a role of retinoic acid (RA) as transcriptional regulator in nerve regeneration. Four lines of evidence support this hypothesis: (1) In various cell culture systems retinoids were found to interact with most cytokine signals that mediate cellular interactions after nerve lesions *in vivo*. (2) Necessary components of the retinoid signaling pathway (aldehyde dehydrogenases, nuclear RA-receptors, cellular RA-binding proteins) are present in the adult nervous system, and glial cells produce RA *in vitro*. In addition, recent observations indicate that RA-synthesizing enzyme activity increases after nerve injury. (3) During development endogenous RA promotes glial and neuronal differentiation including the outgrowth of axons in the developing spinal cord, cerebellum, dorsal root ganglia and sympathetic ganglia. (4) Axonal regeneration of differentiated retinal ganglion cells and peripheral sensory neurons is enhanced by RA *in vitro*.

Introduction

Retinoic acid (RA) is an important morphogen in the development of many organ systems including heart, eyes, hindbrain, spinal cord and urogenital system. All retinoids derive from carotenoids, accessory pigments for photosynthesis in plants. In vertebrates they are oxidized to RA, which acts as the biologically active transcriptional activator (for review see Dräger and McCaffery, 1997; Kochhar, 1997; Durston *et al.*, 1999). In this review I suggest that RA is a regulator of gene transcription not only in embryonic development but also in physiological responses to injuries of the adult nervous system. Especially in the central nervous system (CNS) nerve injuries constitute a problem of clinical relevance: Whereas the transection of a peripheral nerve can be followed by successful restoration of function, lesions in the CNS cause permanent damage. Here, injured nerve cells not only fail to regenerate their axons but suffer degenerative changes and eventually die. Among other causes this difference is due to the fact that the non-neuronal constituents of CNS (astrocytes, oligodendrocytes, microglia) and peripheral nervous system (PNS; Schwann cells, fibroblasts, macrophages) activate different pathways of intercellular signal transduction. In order to understand success or failure of axonal regeneration, it is therefore promising to investigate the cellular interactions that take place in the different environments of CNS and PNS.

An acute injury to the mammalian CNS triggers a complex set of physiological reactions, largely determined by the activation of microglia cells and

Abbreviations: BDNF, brain derived neurotrophic factor; BMP, bone morphogenetic protein; CLF/CLC, cytokine-like factor 1/cardiotrophin-like cytokine; CNS, central nervous system; CNTF, ciliary neurotrophic factor; CRABP, cellular retinoic acid binding protein; DRG, dorsal root ganglion; FGF, fibroblast growth factor; GDNF, glial cell line-derived neurotrophic factor; GF-R, GDNF-receptor; GM-CSF, granulocyte macrophage colony stimulating factor; ICAM, intercellular adhesion molecule; IFN, interferon; IGF, insulin like growth factor; IL, interleukin; Jak, Janus kinase; LIF, leukemia inhibitory factor; N-CAM, neural cell adhesion molecule; NGF, nerve growth factor; NT, neurotrophin; PC-12, rat pheochromocytoma (adrenal gland tumor) cell line; PNS, peripheral nervous system; -R, -receptor; RA, Retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TNF, tumor necrosis factor; Trk, tyrosine kinase (neurotrophin receptor).

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astrocytes, followed by degeneration of nerve cells. (1) Mesodermal microglia cells infiltrate the CNS during early development and remain there in a resting state (Raivich *et al.*, 1999). Activated by a lesion, the microglia approach injured nerve cell bodies, displace synaptic connections and proliferate. The cells then become phagocytotic, attack injured nerve cells and remove cellular debris from degenerating neurons and myelin (Blinzinger and Kreutzberg, 1968; Thanos *et al.*, 1993). (2) Astrocytes, the predominant neuroglial cells of the CNS, also respond to an injury with morphological changes, accompanied by the upregulation of glial fibrillary acidic protein (Graeber and Kreutzberg, 1986). Later, astrocytes *form* a physical barrier between damaged and intact tissue, the glial scar. Together with inhibitory molecules in the CNS-myelin (Caroni and Schwab, 1988; Chen *et al.*, 2000), the glial scar inhibits axonal regeneration (Liuzzi and Lasek, 1987; Puckett *et al.*, 1997). (3) Axotomized nerve cells in the brain react with chromatolytic swelling of the perikarya and neurite sprouting at the site of injury. In the inhibitory environment of the CNS, however, this remains ineffective and is generally followed by cell death. In addition to the glial reaction, the posttraumatic cell death causes infiltration of lymphocytes from the blood vessels (Raivich *et al.*, 1998).

In contrast to the CNS, peripheral nerve fibers are able to regenerate well after a traumatic injury. The physiological responses to the transection of a peripheral nerve consist of two phases: Wallerian degeneration and regenerative growth processes (Mey and Thanos, 1996; Gillen *et al.*, 1997). (1) The term Wallerian degeneration refers to the breakdown and phagocytosis of the nerve segment that is distal to a lesion. Degeneration includes also neuronal cell body reaction, myelin breakdown, cellular infiltration of macrophages from the blood, de-differentiation and proliferation of Schwann cells. (2) The phase of regeneration begins with metabolic changes by the affected nerve cells. Axons then extend from the lesion site, Schwann cells differentiate again and ensheathe the new fibers with myelin. Finally target organs can be re-innervated.

On the molecular level these cellular responses are accomplished by the differential transcription of a large number of structural and regulatory genes. For instance, mRNAs of transmitters and myelin specific genes decline in the degeneration phase, and those of lipid carrier proteins like apolipoproteins D and E increase in the regeneration phase of peripheral nerves (Gillen *et al.*, 1997; Raivich *et al.*, 1999). How are these cellular interactions regulated? To a large extent this is a function of cytokine signaling. Although the differential expression of a large number of cytokine and cytokine receptor genes has been described, much less is known about their transcriptional control. I suggest that RA plays an important role in the regulation of cytokine signaling after nerve injury. To support this hypothesis I will review some facts about differential cytokine expression after nerve lesions, present data on cytokine regulation by RA, and discuss evidence for the relevance of RA in axonal regeneration.

Differential cytokine expression after nerve injury

Cytokines have been described as intercellular mediators that regulate the immune response during infections, inflammatory reactions, and neurological and endocrinological autoimmune diseases. Unlike hormones, cytokines are not stored in glands, then released into the bloodstream, but are synthesized and secreted locally and in relatively small amounts (Heinrich *et al.*, 1998). On the molecular level of description, cytokines are a diverse group of secreted proteins with small molecular weight (6–26 kDa). They are produced by all cell types that participate in the traumatic interactions after a nerve injury. Cytokines initiate their actions by high affinity binding (dissociation constants $< 10^{-10}$ M) to specific cell-surface receptors, which in turn activate intracellular messenger systems, involving protein kinase or phosphatase pathways (Zhao and Schwartz, 1998). After nerve injuries in the PNS (sciatic nerve, facial nerve) or CNS (optic nerve, cerebral cortex) the following cytokines have been discovered to be differentially expressed.

Interleukin-1 (IL-1) family

Most biological functions of these pro-inflammatory cytokines are transmitted by IL-1β. This, but not IL-1α, requires an IL-1-converting enzyme for its activation. In addition to IL-1α and IL-1β a third member of this family is the IL-1 receptor antagonist (IL-1ra), which blocks transmission by

competitive inhibition of their receptor (Gutierrez *et al.*, 1994). Within 1–3 hours after a peripheral nerve lesion IL-1β bioactivity as well as its mRNA levels increase dramatically. In the rat sciatic nerve infiltrating macrophages secrete IL-1β. This binds to Schwann cells and induces the synthesis of nerve growth factor (NGF), granulocyte macrophage colony stimulating factor (GM-CSF) and increases the stability of NGF mRNA (Lindholm *et al.*, 1987; Lindholm *et al.*, 1988). A positive influence on wound repair is also suggested (Streit *et al.*, 1998). In combination with fibroblast growth factor-2 (FGF-2) IL-1α supports neurite outgrowth from dorsal root ganglion explants in three-dimensional astrocyte cultures (Fok-Seang *et al.*, 1998). In contrast to these bona fide beneficial effects, the upregulation of IL-1β in the CNS has been implicated in ischemic, traumatic or excitotoxic damage. Inhibition of IL-1 synthesis or administration of IL-1ra reduced neurodegeneration in these cases (Rothwell, 1998).

Interleukin-6 (IL-6) type cytokines

Leukemia inhibitory factor (LIF), IL-6, ciliary neurotrophic factor (CNTF) and other related molecules are referred to as neuropoietic cytokines and share a common signaling pathway, involving the gp130 cell membrane receptor plus one or two more specific receptors. Ligand binding leads to the activation of gp130-associated kinases (Jak1, Jak2, Tyk2). They phosphorylate gp130 and factors of the STAT family (signal transducer and activator of transcription), which dimerize, translocate to the nucleus, and bind to enhancer elements of respective target genes (Heinrich *et al.*, 1998). Within 24 hours after rat sciatic nerve injury, LIF protein increases in Schwann cells close to the lesion site. In addition to the signaling pathway described above, LIF is retrogradely transported in the axons and then functions as a neurotrophic factor for sensory and motoneurons. In cultured Schwann cells LIF is induced by TGF-β1 (Matsuoka *et al.*, 1997). Neurotrophic properties are also reported for CNTF (Sendtner *et al.*, 1990; Mey and Thanos, 1993). This cytokine is present in large amounts in the cytoplasm of Schwann cells of non-injured nerves. Although expression of the CNTF gene is downregulated due to the injury, cell damage causes release of CNTF from those cells (Sendtner *et al.*, 1992). CNTF, which is also taken up and retrogradely transported, prevents the degeneration of motoneurons (Sendtner *et al.*, 1990). They subsequently become sensitive to LIF (Haas *et al.*, 1999). IL-6 is not detected in the adult PNS but is induced by nerve injury in many large- and medium sized neurons (Murphy *et al.*, 1995). Its expression (as well as that of GM-CSF mRNA) rises dramatically in sheath cells of the injured PNS and CNS. IL-6 has a minor effect on neuronal survival (Murphy *et al.*, 2000), yet supports macrophage activity like myelin breakdown, which in turn stimulates Schwann cell proliferation and axonal regeneration (Gillen *et al.*, 1997). After facial nerve transection, IL-6 participates in the activation of astrocytes and, indirectly, microglia cells (Klein *et al.*, 1997; Raivich *et al.*, 1999). In activated astrocytes, the primary targets of IL-6, this cytokine induces the production of NGF (Kossmann *et al.*, 1996). Recently a new ligand for the CNTF-R has been characterized, a complex consisting of the soluble receptor cytokine-like factor-1 and cardiotrophin-like cytokine (CLF/CLC). This secreted heterodimer supports motor neuron survival and may be responsible for many biological functions of the CNTF-signaling pathway that could not be accounted for in development, because CNTF lacks a signal sequence and is barely detectable in the embryonic CNS (Elson *et al.*, 2000). A possible involvement for CLF/CLC in nerve injury will certainly be investigated. Receptor genes for neuropoietic cytokines in peripheral nerves likewise responds to nerve injury with distinctive expression patterns (Ito *et al.*, 1998; Haas *et al.*, 1999). CNTF-Rα mRNA levels, which are low in the intact nerve, increase gradually during three weeks after nerve crush or transection, then return to normal. LIF-Rβ mRNA concentration is fairly high but shows a transient decrease after the lesions. IL-6-Rα expression increases rapidly within 2 days, then declines steadily. Finally, the gp130 mRNA concentration follows a similar pattern but expression remains high for a longer period, in the regeneration paradigm (crush) for at least 4 weeks and for 2 weeks in the distal part of transected nerves (Ito *et al.*, 1998). The situation appears different for the neuronal component (axons, somata) where receptors for neuropoietic cytokines are expressed constitutively. After transection of the facial nerve CNTF-Rα mRNA and

protein were found to be downregulated, LIF-Rβ upregulated, and gp130 unchanged in the brainstem nucleus of the facial nerve. Intracellular signal transduction (STAT-3 phosphorylation) was activated in the brain (Haas *et al.,* 1999). The neuropoietic cytokines are key players in the mediation of inflammatory reactions. At least three of them, CNTF, CLF/CLC and LIF, exert a positive influence on the homeostasis of injured neurons.

Transforming growth factor-β (TGF-β) family

Different members of the TGF-β family apparently fulfill very different functions. TGF-β1 is an anti-inflammatory cytokine with neurotrophic and immunosuppressive properties. From a low level of expression in the normal sciatic nerve, TGF-β1 is upregulated to reach a maximum concentration within 4 days after injury. TGF-β1 stimulates Schwann cell proliferation and regulates the expression of other downstream genes like neural cell adhesion molecule (N-CAM). On the other hand, TGF-β3 is downregulated immediately after nerve transection, although its mRNA level rises with subsequent axonal regeneration (Rufer *et al.*, 1994; Gillen *et al.,* 1997). Levels of TGF-β2 protein in the lesioned peripheral nerve are low and may derive from the blood (Rufer *et al.,* 1994; Mey and Thanos, 1996). TGF-β1 and TGF-receptor expression also increase after CNS-lesions and in most forms of brain pathology (Raivich *et al.,* 1999). While TGF-β1 deficient mice show a reduction of microglia and an increase in astrocyte activation, exogenous application of TGF-β1 or transgenic overexpression enhanced glial scarring and extracellular matrix deposition (Logan *et al.*, 1994; Hamada *et al.*, 1996; Jones *et al.*, 1998). Thus, TGF-β cytokines exert both beneficial and inhibitory effects on nerve regeneration.

Neurotrophins (NT)

The four neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5, are probably the best known regulators of neuronal survival and connectivity (Bregman *et al.*, 1997; Bartheld, 1998; Kaplan and Miller, 2000). All are differentially regulated after injury (Hefti, 1994; Gillen *et al.,* 1997). Neurotrophin receptors belong to one family of receptor tyrosine kinases (Trk) with specificity for NGF (TrkA), BDNF and NT-4/5 (TrkB), and NT-3 (TrkC), although NT-3 is somewhat promiscuous (Barbacid, 1994). The recently discovered NT-6 and NT-7 also bind to TrkA (Cheung and Ip, 2000). In addition, there exists an unspecific low affinity neurotrophin receptor p75, which has been implicated in NGF-induced cell death (Frade *et al.*, 1996; Frade and Barde, 1998; Kaplan and Miller, 2000). Triggered by a peripheral nerve injury NGF expression goes up in a biphasic response in Schwann cells. Its mRNA level rises transiently after a few hours, drops and increases again after 3 days, remaining high during the phase of regeneration (Heumann *et al.*, 1987; Hengerer *et al.*, 1990). In the sciatic nerve NGF appears to be regulated by IL-1β, though this may be an indirect effect, since IL-1 and tumor necrosis factor-α (TNF-α) can induce IL-6 synthesis, which in the CNS enhances the release of NGF by astrocytes (Zhao and Schwartz, 1998). BDNF shows a similar response as NGF, although in neurons, while it is upregulated in Schwann cells during remyelination several weeks later. In the motoneurons, BDNF expression then decreases concomitant with axonal regeneration (Meyer *et al.*, 1992). NT-3 and NT-4, in contrast, decline in response to the injury. Paradoxically, neurotrophin production also increases in the injured CNS where no regeneration is possible (McKeon *et al.*, 1997). The transcription of neurotrophin receptor genes changes also after nerve transection, which prevents axonal regeneration in the periphery: An increase is observed with TrkB and TrkC in Schwann cells proximal to the lesion, p75 proximal and distal to the lesion. TrkB and TrkC are downregulated in Schwann cells distal to the lesion, p75 in sensory and motoneurons (Gillen *et al.,* 1997). In response to a CNS lesion, astrocytes express the full-length TrkB receptor (McKeon *et al.,* 1997). Of all the intercellular signals, discussed here, the neurotrophins appear to be the most important ones to produce direct effects on neurons.

Other cytokines and synergistic effects

The production of insulin like growth factor (IGF)-I and its receptor increase in parallel with the proliferation of non-neuronal cells. Since IGF-II expression rises later it has been implicated in

axonal regeneration and neuromuscular synaptogenesis. IGF-I has beneficial effects in several neurodegenerative diseases like amyotrophic lateral sclerosis, experimental allergic encephalomyelitis and neuromuscular atrophies (Doré *et al.*, 1997). Treatment of CNS disorders is difficult with IGF, because it cannot pass the blood-brain-barrier. Many other cytokines including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and IL-10 also participate in degenerative and regenerative processes (Gillen *et al.*, 1998; Spera *et al.*, 1998). TNF-α induces macrophage recruitment from the periphery, though it has no direct effect on phagocytosis (Liefner *et al.*, 2000). The discussion above made it clear that a number of synergistic effects exist between various classes of cytokines. Another example, recently discovered, is the reciprocal action between IL-6 and BDNF in adult dorsal root ganglion neurons. Treatment with functional antibodies *in vivo* and results from mice with null mutation of the IL-6 gene suggest that the increase of BDNF after nerve injury in normal mice and rats is caused by this neuropoietic cytokine (Murphy *et al.,* 2000). With cultures of PC12 cells a reverse interaction between neurotrophin and IL-6 is observed: NGF exerts a negative effect on STAT3, whose activation is inhibitory to neurite growth in this cell line (Ihara *et al.*, 1997).

Regulation of cytokine expression by retinoic acid

In embryonic development retinoic acid (RA) regulates glial and neuronal cell differentiation by activating a family of DNA-binding nuclear transcription factors, the retinoic acid receptors (RAR) and retinoid×receptors (R×R) . Among the targets of this transcriptional control are a multitude of structural genes, transcription factors, cell surface molecules and also many cytokines (Gudas *et al.*, 1994). Our knowledge about the influence of RA on cytokine expression derives almost entirely from cell culture experiments, often with immortalized cell lines not related to the nervous system.

The pro-inflammatory cytokines IL-1α and IL-1β are induced by RA in most experiments, whereas the expression of IL-6 is suppressed or enhanced depending on the cell culture system

Table I. Regulation of proinflammatory cytokines by RA.

Cell culture system	Cytokine	RA effect on gene expression	References
Monocytes	**IL-1β**	**suppression**	Gross *et al.*, 1993
Monocytes	**IL-6**	**suppression**	"
Myeloid leukemia cells	**IL-1α**	**induction**	Visani *et al.*, 1996
Lung carcinoma cells Lu-CSF-1	**IL-1β**	**induction**	Ross, 1996
Granulocytes HL60	**IL-1α,β**	**induction**	Grande *et al.*, 1995
Myeloid leukemia cells	**IL-6**	**suppression**	Visani *et al.*, 1996
Myeloid leukemia cells	**GM-CSF**	**suppression**	"
Osteoblasts MC3T3-E1	**IL-6**	**suppression**	Kozawa *et al.*, 1998
Osteoblasts MC3T3-E1	**IL-6**	**inhibition of IL-1 induced synthesis**	Kagechika *et al.*, 1997
Human osteoblasts	**IL-6**	**inhibition**	Ahmed *et al.*, 2000
Granulocytes HL60	**IL-6**	**induction**	Grande *et al.*, 1995
EBV-transformed B-cells	**IL-6**	**induction**	Ballow *et al.*, 1996
Myeloma cells RPMI1640	**IL-6**	**suppression**	Smith *et al.*, 1998
Myeloma cells RPMI1640	**IL-6-R**	**suppression**	"
Neuroblastoma cells SH-SY5Y	**CNTF-R**	**increased synthesis**	Malek and Halvorsen, 1997
Neuroblastoma cells SH-SY5Y	**gp130**	**increased synthesis**	"
Chick embryonic neurons	**CNTF-R**	**induction**	Wang and Halvorsen, 1998
Monocytes	**CNTF-R**	**induction**	"
Monocytes	**M-CSF**	**suppression**	Kreutz *et al.*, 1998
Keratinocytes	**nuclear factor-IL-6**	**antagonism to enhancer action**	DiSepio *et al.*, 1997
Medulloblastoma cells	**LIF**	**suppression**	Liu *et al.*, 2000

Abbreviations: IL, interleukin; GM-CSF, granulocyte monocyte colony stimulating factor; CNTF, ciliary neurotrophic factor; M-CSF, macrophage colony stimulating factor; -R, -receptor; LIF, leukemia inhibitory factor.

(Table I). While the production of IL-1 itself may be supported by retinoids, RA sometimes counteracts downstream effects, for instance the IL-1-induced synthesis of other cytokines (Gross *et al.,* 1993). RA induces the synthesis of CNTF-receptor-α mRNA in embryonic chick ciliary ganglion neurons, and this correlates with an increase in activation of CNTF-dependent intracellular signal transduction, in this case phosphorylation of STAT-3 (Wang and Halvorsen, 1998). Regulation of the various receptors for neuropoietic cytokines depends strongly on the cell culture system with contradicting results even for closely related cell types (Chen *et al.*, 1999). The expression of TGF-β cytokines, which often have a beneficial influence on neuronal survival and regeneration, is generally increased with RA treatment (Table II).

Since the primary function of neurotrophins is seen in the regulation of nerve cell survival and neuronal connectivity, their regulation by RA implies the closest connection between retinoids and nerve regeneration (Table III). Most investigators who demonstrate a supportive effect by RA on axon growth or neuronal survival suggest the transcriptional activation of the neurotrophin receptor genes as the functional mechanism (Kaplan *et al.,* 1993; Holst *et al.,* 1995; Plum and Clagett-Dame, 1996; Ahlemeyer *et al.,* 2000). The importance of RA for neurotrophin receptor expression has been studied most thoroughly in developing sympathetic ganglia of chicken, rat and mouse (Rodríguez-Tébar and Rohrer, 1991; Holst *et al.,* 1995; Holst *et al.,* 1997; Kobayashi *et al.,* 1998; Wyatt *et al.,* 1999). Although there seem to be major differences in the effect of retinoids on expression of receptor tyrosin kinases in sympathetic neuroblasts of birds and mammals, experiments with specific agonists and antagonists suggest that endogenous RA acts on Trk genes via activation of RARα in both classes of vertebrates (Holst *et al.,* 1995; Wyatt *et al.,* 1999).

Glial cell line-derived neurotrophic factor (GDNF) is another type of cytokine involved in neuronal development. Its specific receptor, GFRα1, is upregulated by RA also by way of the nuclear receptor RARα (Thang *et al.,* 2000). In addition to these cytokines, RA affects many different signal transducers with a function in traumatic reactions of the nervous system (Table IV).

Another important pathway of intercellular interaction requires direct cell-cell contact, mediated via cell adhesion molecules of the immunoglobulin superfamily, Ca^{2+}-dependent cadherins, and integrins, the receptors for extracellular matrix molecules (DiProspero *et al.*, 1997; Werner *et al.*, 1998). These are other candidates for targets of transcriptional regulation by retinoids. In F9 teratocarcinoma cells and smooth muscle cells, RA increased the amount of β1 integrin, a laminin/fibronectin receptor subunit. This led to increased adhesion

Table II. Regulation of TGF-β cytokines by RA.

Cell culture system	Cytokine	RA effect on gene expression	References
PC-12 cells	**TGF-β1**	**induction**	Cosgaya *et al.*, 1997
Bronchial epithelial cells	**TGF-β2**	**induction**	Han *et al.*, 1997
Embryonic palate mesenchyme cells	**TGF-β2, β3**	**induction and activation of latent form**	Nugent *et al.*, 1998
Rat prostate epithelial cells NRP-152	**TGF-β1, β2, β3**	**induction**	Danielpour, 1996
Retinal pigment epithelial cells	**TGF-β1**	**suppression**	MacDonald *et al.*, 1995
Bovine endothelial cells	**TGF-β-R**	**induction**	Yoshizawa *et al.*, 1998
Chick osteoclasts	**TGF-β**	**activation of latent form**	Bonewald *et al.*, 1997
Myelo-monocytic leukemia cells U937	**TGF-β1**	**induction**	Defacque *et al.*, 1999
Embryonic testis culture	**TGF-β1, β2, β3**	**induction**	Cupp *et al.*, 1999
Mice *in vivo*, palate	**TGF-β1, β2, β3**	**transient induction**	Degitz *et al.*, 1998
Carcinoma cells P19	**Stra-3/lefty**	**induction**	Oulad-A. *et al.*, 1998
Mouse embryos *in vivo*	**Stra-3/lefty**	**induction**	"
Human pancreatic tumor cells	**TGF-β2**	**induction**	Choudhury *et al.*, 2000

Abbreviations: TGF, transforming growth factor; TGF-R, transforming growth factor receptor.

Table III. Regulation of neurotrophins and neurotrophin receptors by RA.

Cell culture system	Cytokine	RA effect on gene expression	References
Neuroblastoma cells LA-N-1	**NGF-R**	**increased NGF-binding**	Haskell *et al.*, 1987
Neuroblastoma cells	**TrkB**	**induction**	Kaplan *et al.*, 1993; Lucarelli *et al.*, 1995
Neuroblastoma cells	**TrkA**	**mRNA stability**	Lucarelli *et al.*, 1995
Leukemia cells K562, KG-1	**TrkA**	**induction**	Xie *et al.*, 1997
Rat P0 sympathetic neurons	**TrkA**	**suppression**	Kobayashi *et al.*, 1994
Rat P0 sympathetic neurons	**TrkB**	**induction**	"
Chick embryonic sympathetic Neurons	**TrkA**	**induction**	Rodríguez-Tébar and Rohrer, 1991; Holst *et al.*, 1995, 1997
Rat embryonic sympathetic neurons	**TrkA**	**suppression**	Kobayashi *et al.*, 1998
Rat embryonic sympathetic neurons	**TrkC**	**induction**	"
Rat embryonic sympathetic neurons	**NT-3**	**induction**	"
Mouse sympathetic neuroblasts	**TrkA**	**suppression**	Wyatt *et al.*, 1999
Mouse sympathetic neuroblasts	**TrkC**	**induction**	"
Mouse sympathetic neuroblasts	**p75**	**no effect**	"
Rat astrocytes	**NGF**	**induced secretion**	Ahlemeyer *et al.*, 2000
Chick embryonic neurons	**TrkA**	**induction**	Ahlemeyer *et al.*, 2000

Abbreviations: NGF, nerve growth factor; -R, receptor; Trk, tyrosine kinase of the neurotrophin receptor family; NT, neurotrophin; p75, low affinity neurotrophin receptor.

TableIV. Regulation of other cytokines and cytokine receptors by RA.

Cell culture system	Cytokine	RA effect on gene expression	References
Prostate adenocarcinoma cells	**IGFBP-3**	**induction**	Hwa *et al.*, 1997
Prostate adenocarcinoma cells	**IGFBP-5**	**suppression**	"
Hepatoma cells	**IGFBP-1**	**suppression**	Kim *et al.*, 1999
Hepatoma cells	**IGFBP-3**	**suppression**	"
Bronchial epithelial cells	**IGFBP-3**	**induction**	Han *et al.*, 1997
Bronchial epithelial cells	**IL-8**	**induction**	Chang *et al.*, 2000
Hut78 T-cell line	**IL-2-Rα, β**	**induction**	Sidell *et al.*, 1997
Mouse macrophages	**IL-12**	**suppression**	Na *et al.*, 1999
EAE mice	**IL-4**	**induction**	Racke *et al.*, 1995
EAE mice	**IL-2**	**suppression**	"
EAE mice	**TNF-α**	**suppression**	"
EAE mice	**IFN-γ**	**suppression**	"
Embryonic rat sympathetic neurons	**GF-Rα1**	**induction**	Thang *et al.*, 2000

Abbreviations: IL, interleukin; IGFBP, insulin-like growth factor binding protein; EAE, experimental allergic encephalomyelitis; TNF, tumor necrosis factor; IFN, interferon;, GF-R, glial cell line-derived neurotrophic factor receptor.

on laminin (Ross *et al.*, 1994) and fibronectin (Medhora, 2000). A substrate-specific effect of RA on the length of neurites has also been reported for developing olfactory neurons grown on laminin (Whitesides *et al.*, 1998). After facial nerve transection a biphasic upregulation of intercellular adhesion molecule-1 (ICAM-1 or CD54) was observed. The response, which is attributed to activated microglia and in a second phase also to infiltrating lymphocytes, correlates with phagocytotic activity and microglia-lymphocyte interactions (Werner *et al.*, 1998). Again, in an unrelated line of research, RA was found to be involved in the regulation of ICAM-1. When induced by TNF-α the ICAM-1 expression in epidermal keratinocytes was enhanced by RA (Janssens *et al.*, 1999).

Retinoic acid and axonal regeneration

Since RA inhibits proliferation of several immortalized cell lines, many cell culture experiments with retinoids are connected to cancer-research. In these a number of neuroepithelial cell lines can be driven to develop neuronal (Jones-Villenueve *et al.*, 1983; Kaplan *et al.*, 1993; Cheung *et al.*, 2000) or glial (Staines *et al.*, 1996; Tokumoto *et al.*, 1999) characteristics.

RA induces differentiation of neurons and glial cells

During embryonic development of the nervous system RA is an important regulator of cell differentiation, affecting oligodendrocytes (Noll and Miller, 1994; Staines *et al.*, 1996), astrocytes (Wuarin *et al.*, 1990) and nerve cells (Henion and Weston, 1994; Thang *et al.*, 2000). The effect of RA on neuronal differentiation has been studied extensively with embryonal carcinoma cells, where a large number of RA-induced and repressed genes were identified (Bouillet *et al.*, 1995; Oulad-Abdelghani *et al.*, 1998; Cheung and Ip, 2000; Cheung *et al.*, 2000). Neuronal development requires the outgrowth of axons. In cell culture experiments with embryonic tissue RA increased axonal outgrowth from spinal cord (Wuarin *et al.*, 1990; Hunter *et al.*, 1991; Wuarin and Sidell, 1991; Maden *et al.*, 1998), dorsal root ganglia (DRG; Haskell *et al.*, 1987), cerebellum (Yamamoto *et al.*, 1996) and sympathetic ganglia (Rodríguez-Tébar and Rohrer, 1991; Holst *et al.*, 1997). In one study RA exerted a specific effect on BMP-induced dendrite development but not axonal elongation or survival of rat sympathetic neurons. It has therefore been suggested that retinoids function as endogenous morphogens for neuronal cell shape and polarity (Chandrasekaran *et al.*, 2000). If axonal regeneration requires the reactivation of growth-related genes that were used during embryonic differentiation but are quiescent in the adult nervous system, it is not unreasonable to assume that RA, since it plays an important part in developmental regulation of those genes, may again control the metabolic state of injured neurons. A prerequisite for this assumption is the functional machinery of retinoid signal transduction in the adult nervous system (Fig. 1).

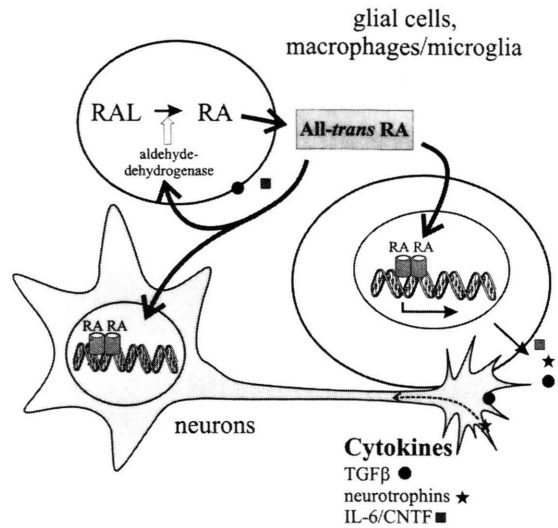

Fig. 1. Retinoic acid as a possible regulator of cytokine signaling in the interactions between nerve cells, glia cells and macrophages/microglia after nerve injury. In this hypothetical scenario RA, synthesized by non-neuronal cells, induces the transcription of neurotrophin receptors in nerve cells and the production of cytokines in Schwann cells or macrophages. At present only indirect evidence for an involvement of retinoids in the physiological reactions after nerve injury is available.

Retinoid signal transduction with retinoids in the adult nervous system

In the vertebrate nervous system RA is synthesized from retinaldehyde, a product of retinol oxidation. The limiting reaction that controls the local availability of RA *in vivo* is the oxidation of retinaldehyde, usually catalyzed by aldehyde dehydrogenases (Napoli, 1996; Dräger and McCaffery, 1997). These enzymes are most active in the embryo, but are also present in the adult nervous system. Neurons, glia cells and the meninges are sources of RA in the embryo and in the adult (Toresson *et al.*, 1999; Yamamoto *et al.*, 1999). Western blotting with an antibody against class 1 aldehyde dehydrogenases demonstrates that such enzymes are expressed in differentiated glia cells. OLN-93 oligodendrocytes are able to oxidize retinaldehyde and specifically synthesize all-*trans* RA (Mey and Hammelmann, 2000). RA is also produced in the adult CNS *in vivo* (Dev *et al.*, 1993), for instance in the retina, the meso-telencephalic dopamine system and the pia mater (McCaffery and Dräger, 1994; Dräger and McCaffery, 1997; Pottek and

Weiler, 2000). Cellular retinoid binding proteins are present in many brain structures: The cellular retinoic acid binding protein CRABP-II has been detected in neurons of the basal forebrain and striatum, CRABP-I in the olfactory system (Zetterström *et al.*, 1999). Intracellular signal transduction of RA depends on ligand activated dimers of nuclear RA receptors. The RXRβ receptor was detected in most areas of the CNS, RXRα in cortex and hippocampus. RARβ and RXRγ are highly expressed in the dopamine-innervated caudate/putamen, nucleus accumbens and olfactory tubercle of adult rodents (Zetterström *et al.*, 1999). Physiological experiments with RARβ/RXRγ knockout mice revealed impairment in spatial learning and memory of these animals (Morris water maze). They also indicate that the RARβ-gene is necessary for long term potentiation and both genes are required for long term depression in the hippocampus (Chiang *et al.*, 1998). This incomplete list demonstrates the presence of various components of the RA system in the adult nervous systems and suggests possible recruitment for signal transduction after traumatic injuries.

RA promotes axonal regeneration in vitro

Until recently, only embryonic neurons were known to respond to RA with neurite outgrowth. Work from my laboratory (Mey and Rombach, 1999) and experiments by Corcoran and Maden (Corcoran and Maden, 1999) have now shown that RA also promotes regeneration of axons by differentiated nerve cells. To investigate the possibility that RA promotes axonal regeneration of chick retinal ganglion cells synergistically with BDNF we injected all-*trans* RA onto the chorioallantoic membrane of stage E16 chick embryos. Following explantation of the retinas 24 hours later, axonal regeneration was monitored in organ cultures supplemented with BDNF. RA enhanced neurite outgrowth of retinal ganglion cells 2- to 3-fold. The dose-dependent and highly significant effect was observed only after application of RA *in ovo* and subsequent use of the neurotrophin. Treatment with RA alone or as a supplement to the culture medium produced no increase in fiber numbers (Mey and Rombach, 1999). With neurons from dorsal root ganglia (DRG), RA seemed to act downstream from a neurotrophin. In this case, NGF caused neurite outgrowth from DRG by activating the synthesis of all-*trans* RA: DRG cultured in the presence of NGF-blocking antibodies and RA showed neurite outgrowth equivalent to that obtained with the neurotrophin alone, whereas the action of NGF was abolished by a blocker of RA-synthesizing aldehyde dehydrogenases (Corcoran and Maden, 1999). The stimulation of neurite outgrowth in NGF- and NT-3-dependent DRG neurons by RA involves specific activation and upregulation of $RARb_2$ (Corcoran *et al.*, 2000). Several cell culture studies show a transcriptional regulation of retinoid receptors by cytokines, e.g. TNF-α (Sugawara *et al.*, 1998). Thus, the causal interaction between retinoids and cytokines may take place in both directions.

Conclusion

The hypothesis that RA plays a role as a regulator of cytokine signaling in the adult nervous system rests on the following lines of evidence:

1. With an interest in their anti-inflammatory and anti-proliferative action retinoids have been thoroughly studied in cell culture systems. In many instances retinoids interact with those cytokine signals that mediate cellular interactions after nerve lesions *in vivo*.
2. Most components of the retinoid signaling pathway are present in the adult nervous system: Aldehyde dehydrogenases, cellular retinoid binding proteins, nuclear RA-receptors. Some of these are upregulated after traumatic injuries.
3. RA promotes glial and neuronal differentiation including the outgrowth of axons in the developing spinal cord, dorsal root ganglia and sympathetic ganglia. Some of these processes are recapitulated in regenerative processes in the injured nervous system.
4. The regeneration of differentiated retinal ganglion cells and peripheral sensory neurons is enhanced by RA *in vitro*.

As in the fields of cancer prevention and dermatological treatment, the developmental role of RA to regulate gene expression opens opportunities for the therapeutic induction of neuronal regeneration.

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