

## CYTOKINES IN IMMUNITY AND INFLAMMATION

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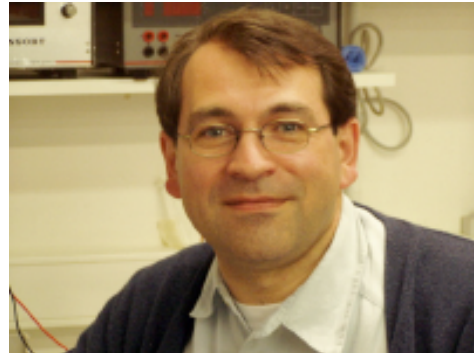
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*The cytokine group studies the biology of Interleukin-9 (IL-9) and IL-22, two cytokines discovered at the Branch. IL-9 is a TH2 cytokine that plays a role in immune responses against intestinal parasites and asthma. IL-22, originally identified as a gene induced by IL-9 in T lymphocytes, upregulates the production of acute phase reagents in the liver. Its activity in inflammatory responses is modulated by a specific antagonist, the IL-22 binding protein (IL-22BP). The role of IL-9 and IL-22 in inflammation is currently being investigated using transgenic and gene-targeted mice for these cytokines and their receptors.*

### Interleukin 9

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Interleukin-9 (IL-9) was discovered in our group in 1989, through its ability to sustain antigen-independent growth of certain murine T helper clones. We further identified human IL-9 by cross-hybridization with the mouse gene. Although IL-9 did not turn out to be a T cell growth factor for freshly isolated T cells, it was found particularly potent on T cell lymphomas, as an anti-apoptotic agent. To determine the biological activities of this factor, we generated transgenic mice overexpressing this cytokine. Analysis of these animals disclosed three essential properties of IL-9: its tumorigenic potential in T lymphocytes, its stimulatory activity on a particular subset of B lymphocytes, and its activity on mast cells and eosinophils with consecutive implications in asthma.

#### **IL-9-transgenic mice : T cell lymphomas**

IL-9 transgenic animals showed normal T cell development and T cell numbers but spontaneously developed thymic lymphomas at low frequency

(5%) when maintained in a conventional environment. Two lines of evidence indicate that IL-9 is not a conventional oncogene but rather favors tumor development in response to exogenous stimuli. First, the tumor incidence was significantly lower when mice were maintained under pathogen-free conditions. Secondly, all IL-9 transgenic mice developed T cell lymphomas when exposed to subliminal doses of a chemical carcinogen or to irradiation, that were innocuous in wild type mice (1). The above mentioned anti-apoptotic activity of IL-9 provides an attractive explanation for these observations, namely that IL-9 could lead to increased survival of abnormal cells generated by exposure to minimal doses of oncogenic stimuli. The potential implication of IL-9 in oncology was also confirmed in human systems by its constitutive expression in Hodgkin lymphomas.

#### **IL-9-transgenic mice : B1 cell expansion**

Further analysis of these IL-9-transgenic mice showed that a particular B lymphocyte population, called B-1 lymphocytes and usually restricted to the peritoneal and pleuropericardial cavities, were dramatically expanded in response to IL-9 overproduction. In addition, such cells were also found in the blood of IL-9 transgenic mice. This observation is reminiscent of mice that are prone to the development of diseases that are characterized by the production of autoantibodies, such as Systemic Lupus Erythematosus, and suggests that

IL-9 might play a role in some autoimmune processes (2).

### ***IL-9-transgenic mice : parasite infections and asthma***

In addition, IL-9 transgenic mice were found to harbor increased numbers of mast cells in the intestinal and respiratory epithelia, and were also characterized by a general hypereosinophilia. This phenotypic characteristic was found to increase the capacity of these animals to expel nematodes like *Trichinella spiralis* or *Trichuris muris*., suggesting that IL-9 administration could protect susceptible hosts against these parasites. This was confirmed by taking advantage of a new strategy of anti-cytokine vaccination: mice vaccinated against their own IL-9 failed to expel *T.muris* parasites and had a decreased eosinophilic response against the parasite (3).

The other side of the coin was the discovery that IL-9 overexpression such as that characterizing the IL-9 transgenic animals resulted in bronchial hyperresponsiveness upon exposure to various allergens. The potential aggravating role of IL-9 in asthma was confirmed by genetic analyses performed by others and pointing to both IL-9 and the IL-9 receptor genes as major candidate genes for human asthma.

### **IL-9 receptor and signal transduction**

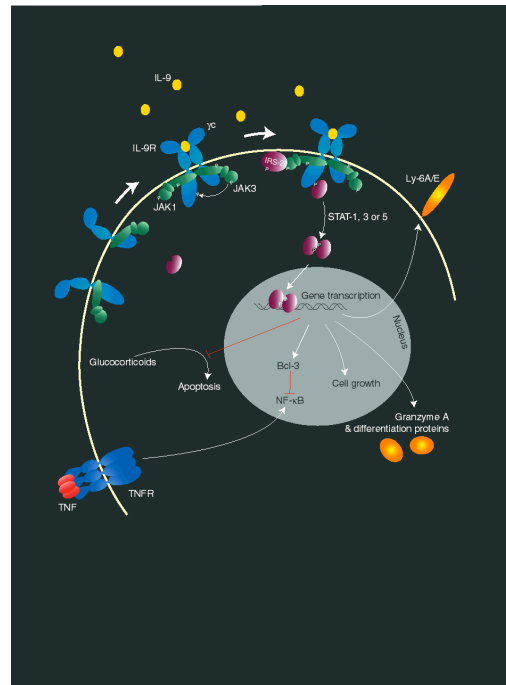
*Jean-Christophe Renault, Laurent Knoops, Diane Lejeune, Monique Stevens, Emiel Van Roost*

Analysis of the mode of action of IL-9 at the molecular level was initiated in 1992 by the cloning of the murine and human IL-9 receptor (IL-9R) cDNAs (4). By further dissecting the signal transduction cascade triggered by IL-9, we showed that, upon IL-9 binding, the IL-9R associates with a co-receptor protein called  $\beta\gamma$ . This induces the phosphorylation of the JAK1 and JAK3 tyrosine kinases, which are associated with IL-9R and  $\beta\gamma$ , respectively. A single tyrosine residue of the IL-9R is then phosphorylated and acts as a docking site for 3 transcription factors of the STAT family, STAT-1, -3 and -5, which become phosphorylated and migrate to the nucleus, where they activate the transcription of a number of genes. This pathway is common to many cytokines but is often dispensable for their biological activities. For IL-9, our group demonstrated that activation of the STAT transcription factors is crucial for all the effects of IL-9 studied on various cell lines, including positive and negative regulation of cell proliferation, as well as inhibition of corticoid-induced apoptosis in T cell lymphomas. Further analysis demonstrated that STAT-1, -3 and -5 play specific, redundant and

synergistic roles in the different activities of IL-9 in vitro (5).

The pathways responsible for IL-9-induced proliferation were studied in details, and this process was found to depend mainly on the activation of STAT-5, on the recruitment of the IRS-1 adaptor, and on the activation of the Erk MAP-Kinase pathway.

The signal transduction pathway downstream the IL-9 receptor is illustrated in Fig. 1.



**Fig. 1**

### **Anti-apoptotic activity of I-309receptor and signal transduction**

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Incidentally, our studies of this particular model of the regulation of cell death by cytokines, lead them to purify another protein called I-309, originally described as a human chemotactic factor, and that turned out to exert a significant anti-apoptotic activity for thymic lymphomas (6). However, I-309 and IL-9 trigger completely different pathways and it was shown that the I-309

anti-apoptotic activity was dependent on the activation of G-proteins and the Ras/MAPKinase pathway, whereas the IL-9-mediated effect was not. More recently, we showed that a viral protein related to human chemotactic factors (vMIP-I), and isolated from Herpes viruses that induce T cell tumors, has the same anti-apoptotic activity by binding to the I-309 receptor

## **IL-9-induced genes**

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To further characterize the mechanisms involved in the anti-apoptotic activity of IL-9 in this experimental model, we sought to identify genes induced by IL-9 in T cell lymphomas. Among the genes we identified, three open unexpected perspectives: BCL3, M-Ras and IL-TIF/IL-22.

### ***BCL3 : indirect modulation of NF- $\kappa$ B***

BCL3 is a gene originally identified at the breakpoint of translocations found in B cell leukemia, resulting in its transcriptional activation. The BCL3 protein interacts with NF- $\kappa$ B transcription factors and its induction by IL-9 represents a novel mechanism of NF- $\kappa$ B regulation by cytokines, and a new crosstalk between the JAK/STAT and NF- $\kappa$ B signal transduction pathway (7). BCL3 induction might play a role in the antiapoptotic activity of cytokines such as IL-4 and IL-9.

### ***M-Ras : transcriptional regulation of the Ras-MAPKinase pathway***

M-Ras is a new member of the Ras oncogene superfamily. The Ras proteins are known to regulate various cellular processes such as proliferation and apoptosis, when they are in their activated form, in association with a GTP molecule. Contrasting with the potent upregulation of M-Ras expression, M-Ras was not activated by IL-9 at the level of GTP binding. However, other cytokines such as IL-3 increased GTP binding to M-Ras, suggesting that M-Ras induction might represent a new mechanism of cooperativity between cytokines. Constitutively activated M-Ras mutants trigger the MAP Kinase pathway and induce proliferation of cytokine-dependent cells (8).

### ***IL-TIF/IL-22 : a new cytokine structurally related to IL-10***

IL-TIF is a new gene that turned out to encode a 179 amino acid long protein, including a potential

signal peptide, and showing a weak but significant sequence homology with IL-10. This protein, originally designated IL-TIF for IL-10-related T-cell derived Inducible Factor, was later renamed IL-22. Its expression is induced by IL-9 in thymic lymphomas, T cells and mast cells and by lectins in freshly isolated spleen cells. In addition, constitutive expression of IL-22 was detected by RT-PCR in thymus and brain, suggesting that the role of this new factor is not restricted to the immune system. Preliminary experiments showed that IL-22 induces STAT activation in various cell lines, suggesting that this factor might mediate some of the activities of IL-9. Biological activities of IL-22 include the induction of acute phase proteins in liver (9). Recombinant human IL-22 was produced (with D. Colau, LICR) and its crystallographic structure solved. Despite its structural homology with IL-10, IL-22 fails to recapitulate any of IL-10 biological activities.

Analysis of the genome databases leads to the identification of a new receptor belonging to the IL-10 receptor family (10). This gene is located in the chromosome 6q24, at 24 kb from the IFNGR1 gene and at 152 kb from the IL-20R. It encodes a protein of 231 amino acid, showing 33 % and 34 % amino acid identity with the extracellular domains of the IL-22R and the IL-20R, respectively, but no cytoplasmic nor transmembrane domains were found. IL-22BP is highly expressed in the placenta, in the breast, in the mammary gland and in the skin. A specific interaction was demonstrated between insolubilized IL-22 and an IL-22BP-Ig fusion protein. Moreover, recombinant IL-22BP could block IL-22 biological activity demonstrating that this protein can act as an IL-22 antagonist.

Although IL-22 does not share any biological activity with IL-10, these 2 cytokines share a common component of their respective receptor complex, IL-10R $\beta$ . Anti-IL-10R $\beta$  antibodies indeed block the IL-22-induced acute phase response in HepG2 cells (9). All receptor complexes for IL-10-related cytokines include a long chain and a short chain, based on the length of the cytoplasmic domain of these transmembrane proteins. IL-10R $\beta$  is a typical short chain component, with only 76 amino acids in the cytoplasmic domain, whose main function seems to consist in recruiting the Tyk2 tyrosine kinase. In addition to IL-10R $\beta$ , IL-22 signalling requires the expression of a long chain protein, called IL-22R and comprising a 319 amino acid long cytoplasmic domain. This chain associates with Jak1, and is responsible for the activation of cytoplasmic signalling cascades such as the JAK/STAT, ERK, JNK and p38 MAP kinase pathways.

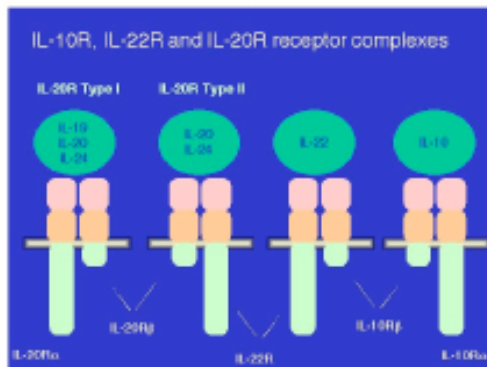


Fig. 2

In addition to its role in IL-22 binding and signalling, the IL-22R chain also forms a functional heterodimeric receptor complex by associating with IL-20R  $\beta$ , the second short chain member of the IL-10R-related receptor family. This complex mediates STAT-1 and -3 activation by IL-20 and IL-24, but not by IL-22 (11). In addition, IL-20 and IL-24 can also bind to other complexes consisting of IL-20R $\alpha$  and IL-20R $\beta$ . This promiscuity in cytokine receptor usage is illustrated in Fig 2.

### Selected publications

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