

## PI-9 and antigen presentation

with

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### What is known about the proteinase inhibitor 9?

**Carl Friedrich Classen:** Proteinase inhibitor 9 (PI-9/SERPINB9) is a 42 kDa human serine proteinase inhibitor that - by formation of a stable complex with 1:1 stoichiometry - irreversibly inactivates granzyme B (GrB), the most prominent proteolytic enzyme in the cytolytic granules of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells (1-5). PI-9 is the only known endogenous natural inhibitor of GrB, and PI-9 overexpressing cells acquire protection from GrB-dependent, but not CD95-dependent cytotoxicity (2). PI-9 is expressed in the cytoplasm and nuclei especially of cytotoxic lymphocytes (T and NK cells), antigen presenting cells (dendritic cells, macrophages, B cells), and cells at immunoprivileged sites, like the placenta, the testis, the ovary, and the eye (4-7). High expression of PI-9 has also been observed in EBV-transformed lymphoblastoid cell

lines (1,8). Regulation of PI-9 expression involves nuclear factor-kappa-B (NF-kappaB) and activating protein-1 (AP-1) responsive elements in the promoter of PI-9 (9), and further, a unique downstream estrogen-responsive element has been shown in hepatocytes (10). PI-9 expression upon cytokine treatment has been studied by several authors including ourselves (8,9,11). We found that in monocytes, IL-2 and lipopolysaccharide induced a marked upregulation of PI-9, which was inhibited by dexamethasone and by an NF-kappaB inhibitor (8). In a similar way, upregulation has been shown upon stimulation of dendritic cells (11). In lymphocytes, data are inconclusive. While others and ourselves (8,12) did not find protein upregulation upon short and long term stimulation, using high doses of cytokines, upregulation can be seen in some subsets (7).

### Have you observed an upregulation of PI-9 in patients?

**Carl Friedrich Classen:** Study of PI-9 in patients are interesting in two fields - in oncology, where PI-9 may contribute to resistance of malignant cells to cytotoxic kill, and in immunology, where PI-9 may be involved in regulation or dysregulation of the immune response.

Studies on PI-9 expression in malignomas have shown inhomogeneous overexpression in a number of disorders, especially lymphomas (5,11,13). In the main subtypes of pediatric acute lymphoblastic leukemias we found a differential expression, however, it was not correlated to a certain leukemia subtype (14). Further, the meaning of PI-9 for immune resis-

tance or as prognostic marker still is open, since some studies do (15), and others do not find a correlation (13,14).

Analysis of PI-9-expression in normal cells of patients suffering from infections or immune regulation disorders may help to understand its normal role in immune homeostasis. Such analyses are, however, rare. Muthukumar et al. (16) found an elevated urine excretion of PI-9 RNA in kidney transplant rejection, indicating PI-9 upregulation as part of the immune reaction. We analysed PI-9 expression in the lymphocyte and monocyte fraction from patients under different clinical conditions (8). In

bacterial infections in older children or adults, PI-9 was unchanged in monocytes, but decreased in lymphocytes. Acute EBV infection and GVHD were associated with PI-9 upregulation, which was stronger in the monocyte than in the lymphocyte fraction. This was not restricted to a specific subset of lymphocytes.

Analysis of preterm infants showed a marked downregulation upon bacterial infection, both in lymphocytes and in monocytes (8). To my knowledge, not many other studies exist analysing regulation of PI-9 in normal cells of patients suffering from infections or immune regulation disorders.

### Why do you believe that PI-9 might be involved in the regulation of antigen presentation?

**Carl Friedrich Classen:** Since PI-9 is specifically regulated in monocytes and antigen presenting cells (8,11), a role in their function is not unlikely. However, if the main function of PI-9 is inhibition of GrB, first, the role of GrB in immune regulation has to be defined. Recent data show that besides acting on malignant or infected cells that are targets of the immune response, GrB also acts on the NK cells and CTL themselves by leaking out of the cytotoxic granula upon activation, thus providing a mechanism of activation induced cell death (17,18,19). A similar role of cytolytic granule enzymes for downregulation of antigen presenting cells (APC) is further indicated by the fact that defects of granular cytotoxicity lead to syndromes of uncontrolled activation not only of the lymphocytic, but also the histiocytic system, like the hemophagocytic lymphohistocytosis syndrome observed in perforin deficiency (20).

A role of PI-9 in the interplay between lymphocytes and APC has been proposed by several authors (8,11). The most interesting study in this field was done by Kim et al. (21). The authors explored intradermal coadministration of DNA encoding the mouse PI-9 homolog SPI-6 with DNA constructs encoding human papillomavirus type 16 E7 for their ability to generate E7-specific immune responses. This resulted in significantly increased E7-specific CD8 T-cell and CD4 Th1-cell responses when compared with vaccination without SPI-6 DNA. The authors concluded that coexpression of SPI-6 lead to enhanced and prolonged presentation of the antigen thus enabling a stronger immune response.

In view of this, let me give my personal speculation. If in lymphocytes, PI-9 protein expression is relatively constant despite stimulation, while in monocytes or APC, it is upregulated, this may transmit different functions in homeostasis regulation: long-term survival of inactive, but short-term survival of activated cytotoxic lymphocytes, and, in contrary, long-term survival of stimulated, but suppression of unstimulated APC. This regulatory circle may provide a rapid and strong immune response on one hand, but on the other hand protect from inflammatory over-reactions, unless the antigen is continuously acting in the system (8).

If PI-9 indeed was a key regulator of immune response, it would be a very interesting molecule to be addressed therapeutically - to enhance immune reaction against malignant cells or infectious pathogens on one hand, or to help turning down an deleterious immune response, like, for example in certain autoimmune disorders

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