The "immunological homunculus" and the "connectivity fingerprint"

It is our conviction that, if autoimmune pathology represents a deviation of physiological autoreactivity, and the latter is reflected in the repertoires of natural serum antibodies, it should be possible in principle to detect such alterations before the pathological process has led to irreversible damage of the target tissues or functions. We have therefore invested a considerable effort into the development of techniques that would provide us with global analyses of the serum antibody repertoires. This required the utilisation of large panels of antigens (several hundreds of homologous and heterologous proteins), automatic data processing, and the application of multiparametric statistics. In parallel, we have attempted to develop techniques for the quantitative description of the connectivity of serum antibodies to a normal and representative set of V-regions. The results of the observations so far conducted in humans, rats and mice indicate that natural IgM and IgG antibody repertoires are stringently selected from the available repertoires in the same individuals. Furthermore, beyond the genetic heterogeneity in the populations analysed, there is a striking conservation in the autoantibody reactivity patterns of adult individuals in the same species, which is almost complete in the newborn stage. The set of antigens recognised thus defines an "immunological homunculus" which is the distorted "representation" of the molecular self-composition by the normal immune system. In contrast, natural antibody reactivities towards heterologous antigens are more variable between individuals, a variability which increases with age. This supports the notion that the immune system starts by the expression of a "programmed" set of genes that encode self-reactive antibodies, which represent the most "selected" set of reactivities throughout life. Interestingly, the analyses of sera from donors afflicted by several autoimmune conditions reveal characteristic alterations in the global patterns, such that these methods may well provide the means for their diagnosis and follow-up.

Activation and recruitment of neutrophil leukocytes

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Neutrophils are the most frequent immigrant cells in inflammatory lesions, and major effectors of tissue damage through their neutral and acid proteases and their ability to generate reactive oxygen derivatives. Circulating neutrophils are quiescent and must be actively recruited, by chemotactic agonists, into the affected tissue, a process involving multiple activation steps. The mechanism of chemotaxis and neutrophil activation began to unravel with the work on *N*-formylmethionyl peptides and on the complement fragment C5a. A breakthrough came about in recent years with the discovery of interleukin-8 (IL-8) and several related chemotactic cytokines (chemokines) which are long-acting, show selectivity for different leukocytes and are produced in many different tissues [1,2].

All chemoattractants elicit in neutrophils a characteristic pattern of responses, including activation of the motile apparatus, directional migration, expression of surface adhesion molecules, exocytosis from different storage organelles, and the respiratory burst [3]. The mechanism of activation has been studied extensively, as documented by a number of reviews from our laboratory [3-6] Two elements are believed to be essential for neutrophil activation and, in particular, for the process of transduction of chemotactic agonist signals, a rise of the cytosolic free calcium concentration ($[Ca^{2+}]_i$) and the activation of protein kinase C. Responses depend on the ongoing interaction of the agonist with its receptor [4], the interaction between the receptor and a GTP-binding protein and the activation of phosphatidylinositol-specific phospholipase C. This enzyme delivers the products which bring about the translocation of protein kinase C to the plasma membrane and its subsequent activation. The fungal metabolite wortmannin is the most potent inhibitor of neutrophil activation [7]. It was recently found that wortmannin selectively blocks PI3 kinase, demonstrating that this enzyme is essential for the induction of exocytosis and the respiratory burst [7,8].

The chemokines are small proteins consisting of 70 – 80 amino acids with four conserved cysteines forming two essential disulphide bonds, a short amino-terminal and a relatively long carboxyl-terminal domain. Two subfamilies are distinguished according to the arrangement of the first two cysteines which are either separated by one amino acid (CXC chemokines) or adjacent (CC chemokines). CXC chemokines act mainly on neutrophil leukocytes, while CC chemokines are inactive on neutrophils, and stimulate monocytes, basophil and eosinophil leukocytes, and T lymphocytes. The genes of the two chemokine subfamilies are clustered on different chromosomes, chromosome 4 for the CXC and 17 for the CC chemokines [1,5].

Since this paper is focused on neutrophils, only CXC chemokines will be considered. After the discovery of IL-8 several analogues were identified in rapid succession: neutrophil-activating protein-2 (NAP-2), which arises from the *N*-terminal processing of platelet basic protein, three GRO proteins (GROα, GROβ and GROτ) and an epithelial cell-derived neutrophil-activating protein, ENA-78 [1].

Selective receptors for IL-8 were demonstrated by binding studies and were shown to be coupled to GTP-binding proteins. Human neutrophils possess high numbers of such receptors (64,500 ± 14,000 on average in our studies) an apparent K_d of 0.18 ± 0.07 nM [9,10]. Radiolabelled IL-8 is displaced by cold IL-8. Displacement by NAP-2 and GROα was found to be bimodal, revealing the existence of two types of receptors: One with high affinity for all CXC chemokines, and the other with high affinity for IL-8 only [9,10]. The receptor which is selective for IL-8 is termed IL-8R1 or IL-8RA, and the other IL-8R2 or IL-8RB [1]. The cDNAs of two receptors for IL-8 were cloned and shown to code for seven-transmembrane-domain receptors [11,12]. These results confirmed the evidence provided by functional studies that IL-8 and other CXC chemokine act via rhodopsin-type receptors like all other neutrophil chemotaxins known [1].

It was originally believed that IL-8 interacts with its receptors through its carboxyl-terminal α-helix revealed. Using synthetic IL-8 analogues, we found that removal of the entire carboxyl-terminal sequence after the fourth cysteine decreased but did not suppress the biological activity of IL-8. In contrast, receptor binding and neutrophil activation were abrogated by deletion [13] or by substitution [14] of the amino-terminal sequence Glu-Leu-Arg (ELR), indicating that this motif is essential for receptor recognition and signalling. Interestingly, the ELR motif is common to all CXC chemokines that activate and attract neutrophils, but is not found in other chemokines. A direct demonstration that the ELR motif is essential for CXC chemokine activity on neutrophils was obtained by substituting ELR for the natural DLQ sequence in platelet factor 4 (PF4). ELR-PF4 competes for IL-8 binding, and induces chemotaxis and enzyme release responses in

neutrophils similar to those observed with IL-8. On the other hand, the truncated form of PF4 (DLQ-PF4) as well as IL-8 with ELQ in place of ELR were inactive [15].

After recognition of the importance of the *N*-terminus, several analogues of the amino-terminally truncated IL-8 were synthesized as potential IL-8 receptor antagonists. Deletions or amino-acid replacements in the ELR region led to the envisaged goal. The most potent antagonists identified so far are R-IL-8 and AAR-IL-8. They inhibit IL-8 receptor binding, exocytosis (IC_{50} 0.3 μM], hemotaxis and the respiratory burst. Inhibition is restricted to responses elicited by IL-8, GROα or NAP-2, and no effect is observed when the unrelated agonists fMet-Leu-Phe or C5a are used as stimuli, demonstrating that they are selective for IL-8 receptors [16].

Since neutrophils always express high numbers of both IL-8 receptors, it was interesting to explore the function of the single species. IL-8R1 and IL-8R2 were, therefore, expressed separately in Jurkat cells. Challenge with IL-8 or GROα showed that both receptors function independently and mediate the same pattern of responses to different CXC chemokines [17].

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Granules and secretory vesicles of the human neutrophil

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Introduction

In order to fulfill its role in host defence against invading microorganisms, the human neutrophil is equipped with a battery of proteases and bactericidal substances and has the ability to assemble an electron transport chain (the NADPH oxidase) capable of generating huge amounts of reactive oxygen species, all contributing to the bactericidal and tissue destructive armory of this cell. As a mobile phagocyte, the neutrophil must circulate throughout the entire vascular bed without compromising the integrity of the microcirculation, yet, at sites of incipient inflammation, the cell must be able to adhere rapidly to endothelium, diapedese and migrate into tissues to phagocytose and kill offending microorganisms. Thus, the neutrophil must be able to adjust rapidly to changes in its surroundings in order to meet the requirements necessary for optimal function in the inflammatory reaction.

The neutrophil is equipped with a plethora of discrete granule subsets and vesicles each with their characteristic protein profile (Table 1). The strictly hierarchic mobilization of these [1,2], offers a structural basis for orchestrating the step-wise metamorphosis of the neutrophil from a stage of circulating quiescence to a stage of maximal metabolic and destructive activity, characteristic of neutrophils in the inflammatory focus.