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Quantifying the relevance of different mediators in the human immune cell network

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ABSTRACT

Motivation: Immune cells coordinate their efforts for the correct and efficient functioning of the immune system (IS). Each cell type plays a distinct role and communicates with other cell types through mediators such as cytokines, chemokines and hormones, among others, that are crucial for the functioning of the IS and its fine tuning. Nevertheless, a quantitative analysis of the topological properties of an immunological network involving this complex interchange of mediators among immune cells is still lacking.

Results: Here we present a method for quantifying the relevance of different mediators in the immune network, which exploits a definition of centrality based on the concept of efficient communication. The analysis, applied to the human IS, indicates that its mediators differ significantly in their network relevance. We found that cytokines involved in innate immunity and inflammation and some hormones rank highest in the network, revealing that the most prominent mediators of the IS are molecules involved in these ancestral types of defence mechanisms which are highly integrated with the adaptive immune response, and at the interplay among the nervous, the endocrine and the immune systems.

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INTRODUCTION

Network analysis has emerged as a powerful approach to understand complex phenomena and organization in social, technological and biological systems (Albert and Barabasi, 2002; Dorogovtsev and Mendes, 2003; Wasserman and Faust, 1994; Editorial, 2002). In particular, the role played by the topology of cellular networks, the intricate web of interactions among genes, proteins and other molecules regulating cell activity, in unveiling the function and the evolution of living organisms is increasingly being recognized (Jeong *et al.*, 2000, 2001; Wagner and Fell, 2001; Maslov and Sneppen, 2002; Milo *et al.*, 2002). The cells of the immune system (IS) have various ways to communicate with each other: directly, by establishing bounds between cell surface ligands and receptors, and indirectly, by means of a variety of soluble mediators released and bound by the immune cells (Abbas *et al.*, 2003; Janeway *et al.*, 2001). Soluble mediators implement cellular communication both at short range (autocrine and paracrine cell stimulation) and across the major body systems (immune, endocrine and nervous systems). These mediators have a fundamental role in regulating the reaction of the IS to a possible danger, which triggers an integrated response involving both innate and clonotypic immunity, and which eventually results in an inflammatory response. Mediators are characterized by pleiotropy (each mediator has multiple targets) and redundancy (each mediator is produced by several sources), two characteristics that strongly influence the reliability of the IS and that significantly contribute to its robustness and adaptability. From an experimental as well as theoretical point of view, the main attention has been generally focused on a few limited subsets of immune cell types and mediators. Here we follow a different approach to understand the global properties of the IS network by modelling the whole system of immune cells as networked by soluble mediators.

SYSTEMS AND METHODS

The network we consider comprises various immune cell types which can act as both sources and targets of the exchanged mediators.

We built a network of IS cells interactions. In this view, the cell's role is uniquely that of an element in a network of cell interactions. We consider these interactions mediated by soluble molecules such as cytokines, chemokines and hormones (Fig. 1).

From experimental data we have two types of information: (1) each cell type secretes a defined set of mediators; (2) each mediator affects a defined set of cells. Combining these two datasets we can write down a complete 'relationship matrix' among cell types where the relations are constituted by the exchanged soluble mediators: e.g. the B lymphocyte cell type secretes TGF-β that affects the dendritic cell type. In this way there is a directional interaction between the B lymphocyte cell type and the dendritic cell type: B cell → dendritic cell. Often the interaction between two cell types is mediated by more than one mediator.

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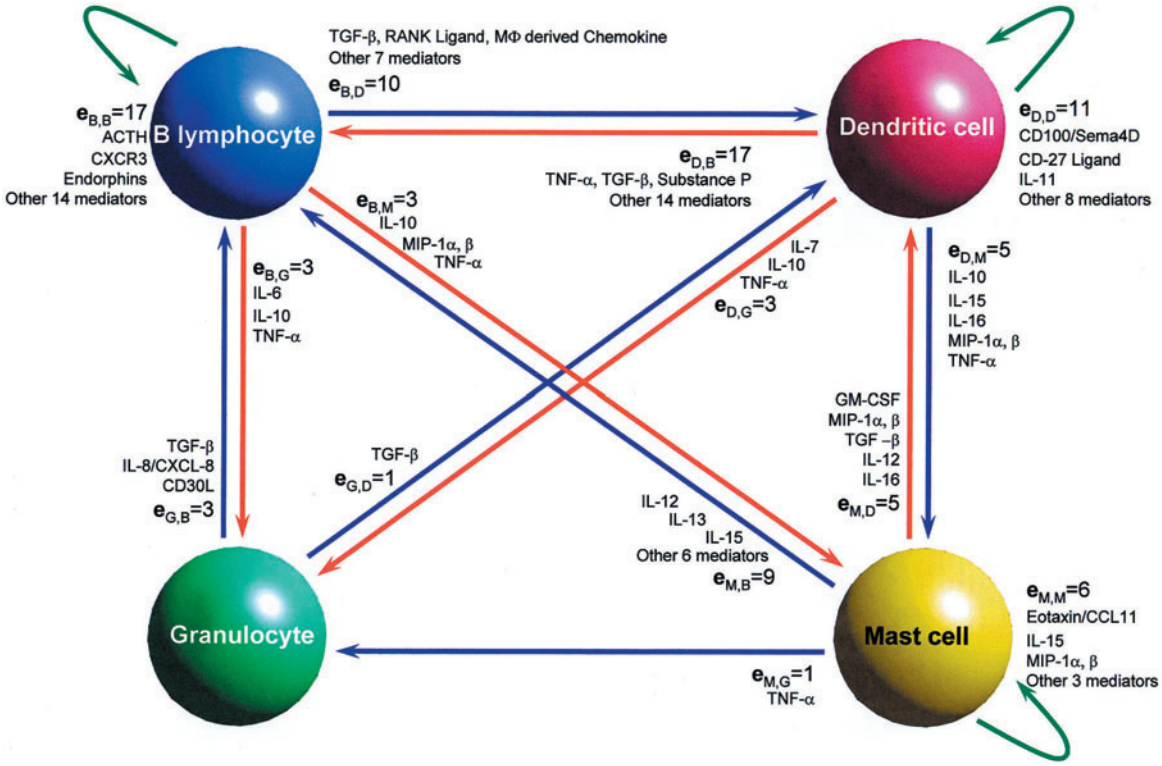

Fig. 1. Portion of the immune cell network considered. In the figure a network with only four out of the 19 cell types considered is illustrated. Cell types are the vertices of the graph, while diffusible mediators, which enable the cells to communicate with each other, define the arcs of the graph. Autocriny is also considered and depicted in the figure. The value e_{ij} associated with each arc is equal to the number of different mediators connecting cell i to cell j. The names of the mediators are listed close to the respective arcs. The 19 cell types considered in the network are: B lymphocytes, basophils, dendritic cells, endothelial cells, eosinophils, epithelial cells, fibroblasts, granulocytes, macrophages, mast cells, monocytes, neutrophils, NK cells, spleen cells, stromal bone marrow cells, T lymphocytes, T CD4+ lymphocytes, T CD8+ lymphocytes and thymocytes. B, B lymphocytes; D, dendritic cell; G, granulocyte; M, mast cell; MΦ, macrophage.

Following this approach we retrieved all available literature data from the online Cytokine Reference Database (Oppenheim *et al.*, 2000, http://apresslp.gvpi.net/apcyto/lpext.dll?f=templates&fn=main-h.htm&2.0), and we chose a wide set of 19 cell types involved in the most relevant immune processes and the related secreted and affecting mediators (90 molecules among cytokines, chemokines and hormones).

IMPLEMENTATION

The immune cell network is represented as a valued directed (Wasserman and Faust, 1994) graph G, where the N cell types considered are the vertices of the graph and the M soluble mediators form its K arcs: a directed arc from vertex i to vertex j is defined by the existence of at least one mediator secreted by cell i and affecting cell j. Cell self-stimulation by soluble mediators (autocriny), which is an important peculiarity of the immune cell network, is also taken into account. The value e_{ij} attached to the arc is assumed to be equal to the number of different mediators connecting cell i to cell j (Fig. 1). We consider such a number as a measure of the importance of the communication between two cells along the arc, hence modelling this as an efficiency (Latora and Marchiori, 2001) that measures the bandwidth.

The IS is basically a parallel working system: all cells concurrently send information along the network through their outgoing arcs, i.e. by secreting mediators, and receive information through their incoming arcs, i.e. by binding the mediators. Two cells/vertices in G can communicate through various paths, connecting them with different levels of efficiency: the efficiency of a path is the harmonic composition of the efficiencies of the component arcs (Crucitti *et al.*, 2004). The harmonic composition of p numbers $x_1, x_2, \ldots, x_p$ is defined as $(\sum_{i=1}^{p} 1/x_i)^{-1}$. We assume that the communication between vertices i and j takes the most efficient path, and the efficiency of such a path is indicated by ε_{ij}. Matrix $\{\varepsilon_{ij}\}$ is computed from the adjacency matrix $\{e_{ij}\}$ by means of the Floyd-Warshall algorithm for the all shortest paths problem. This approach represents an extension to valued networks of the shortest path assumption commonly used in social (Wasserman and Faust, 1994), biological (Albert and Barabasi, 2002; Jeong *et al.*, 2000, 2001; Wagner and Fell, 2001) and communication/transportation (Albert and Barabasi, 2002; Dorogovtsev and Mendes, 2003; Latora and Marchiori, 2001) networks. We characterize the global properties of the system by defining the network efficiency as (Latora and Marchiori, 2001):

$$E(G) = \frac{1}{N^2} \sum_{i,j \in G} \varepsilon_{ij}$$

Network analysts have used centrality as a basic tool for identifying key individuals in a network (Wasserman and Faust, 1994).

A variety of measures of centrality have been proposed over the years to quantify the topological importance of a node in a graph (Wasserman and Faust, 1994; Freeman, 1979; Latora and Marchiori, 2004a,b). Here we propose a method for quantifying the centrality of the various soluble mediators in the IS. The method is based on the concept of efficient communication over the immune cell network. The centrality of each mediator $\alpha(\alpha = 1, \ldots, M)$ is measured by its network relevance r_α, defined as the relative drop in the network efficiency E caused by the removal of the mediator, namely:

$$r_\alpha = \frac{E(G) - E(G'_\alpha)}{E(G)} \quad \alpha = 1, \ldots, M$$

where G'_α is the graph obtained by removing mediator α from G. In fact, in our framework, the removal of a mediator weakens some of the values e_{ij} attached to the arcs and, consequently, affects the communication between various couples of cells, decreasing some of the ε_{ij} and thus the network efficiency of the IS.

New emerging knowledge in immunology can easily be included in our analysis. Newly discovered mediators, new knowledge on mediators' cell secretion and/or on their influence on cells could change the topology of the network and perhaps its global properties as well as the relevance of the mediators.

RESULTS AND CONCLUSION

The method has been applied to human data retrieved by the Cytokine Reference Database (Oppenheim *et al.*, 2000), taking into account $N = 19$ immune cell types (listed in Fig. 1) and $M = 90$ soluble mediators (listed in Fig. 3) selected from among all the available mediators that connect at least two different cell types from our list of immune cells. The resulting graph G is dense, having $N = 19$ vertices and $K = 316$ arcs (including self-connections) out of the 361 arcs of the full graph, and thus a pure topological analysis would be poorly significant. Therefore, we performed a more refined analysis by taking into account the strength of the interactions among the IS cells. The integer values e_{ij} attached to the arcs range from 1 to 36, since there are up to 36 different mediators connecting a couple of cells. In Figure 2 we plot the cumulative relevance distribution defined as $P(r) = M(r)/M$, where $M(r)$ is the number of mediators with relevance larger than r. For large values of the relevance, $P(r)$ shows a power-law behaviour $P(r) \approx r^{-(\gamma-1)}$ with a scaling exponent $\gamma = 2.8 \pm 0.1$.

The fat tail in the mediators' relevance distribution indicates that the universal scaling principles discovered in other biological networks (Albert and Barabasi, 2002; Dorogovtsev and Mendes, 2003; Jeong *et al.*, 2000, 2001) also seem to be fundamental ingredients of the human IS architecture. This shows that the concept of importance is selective and radical: immune cells form a highly inhomogeneous network in which a few soluble mediators play a central role in mediating the interactions between the different cell types.

The network relevance of each mediator is reported in Figure 3. The plot shows that only three mediators, TGF-β, MIP-1-α and -β (grouped as one mediator; Oppenheim *et al.*, 2000) and TNF-α have network relevance >0.5; 11 mediators have network relevance in the range [0.2, 0.5]; and, the remaining 76 have network relevance in the range [0, 0.2]. The sum of the network relevance of the first three mediators accounts for 20.5% of the total mediators' relevance, while those of the second and third groups account for 27.6% and 51.9%, respectively. The unequal role played by the mediators in the

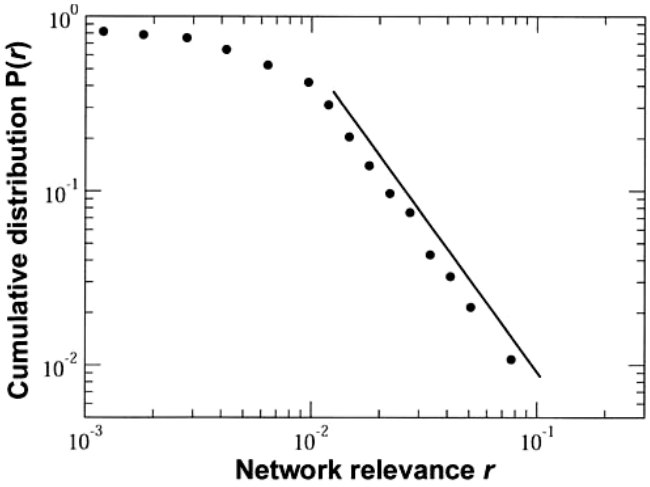

Fig. 2. Distribution of the number of mediators having a network relevance larger than r. The relevance of a mediator is calculated as the relative drop in network efficiency caused by its removal from the network. To reduce noise, logarithm binning was applied. The linear tail in the figure indicates a scale-free power-law behaviour in the relevance of the various mediators of the immune cell network that can be fitted with a curve $P(r) \cong r^{-(\gamma-1)}$ with $\gamma = 2.8 \pm 0.1$.

efficient coordination of the immune network can be quantified by means of the Gini coefficient. The Gini coefficient g is a measure used in economics and ecology to describe size-wealth inequality in a population. It compares the Lorenz curve of a ranked empirical distribution, i.e. a curve that shows, for the bottom x% of individuals, the percentage y% of the total size which they have, with the line of perfect equality (Dagum, 1980). The coefficient g ranges from a minimum value of zero, when all individuals are equal, to a theoretical maximum value of 1 in a population in which every individual except one has a size of zero. The value we have found $g = 0.64$ confirms the large skew of relevance.

The three most important mediators are pro- and anti-inflammatory molecules and are involved in the communication among a large number of cell types, i.e. in 216, 224 and 120 interactions, respectively. The second group of mediators includes nine pro- and anti-inflammatory cytokines/chemokines, one non-inflammatory cytokine (IL-7) and one neuroendocrine hormone (VIP/PACAP). Therefore, notwithstanding the fact that mediators involved in the inflammatory process account only for 24% of all mediators, 86% (12 out of 14) of the top molecules are inflammatory, accounting for 50% of all inflammatory mediators.

The analysis shows that mediators involved in innate immunity and inflammation have the most central role in the immune network. In the last decade, innate immunity and inflammation have emerged as central components of the capability of the IS to sense 'danger' signals and to start immune responses, in turn involving cells of adaptive immunity (Matzinger, 2002; Medzhitov and Janeway, 2002). The importance of innate immunity and inflammation is reinforced by recent data indicating that survival at extreme ages and conversely mortality caused by major age-associated diseases are related to low or high level of inflammation ('inflamm-aging', Franceschi and Bonafè, 2003; Abbott, 2004), respectively. From this point of view it is challenging that several inflammatory mediators,

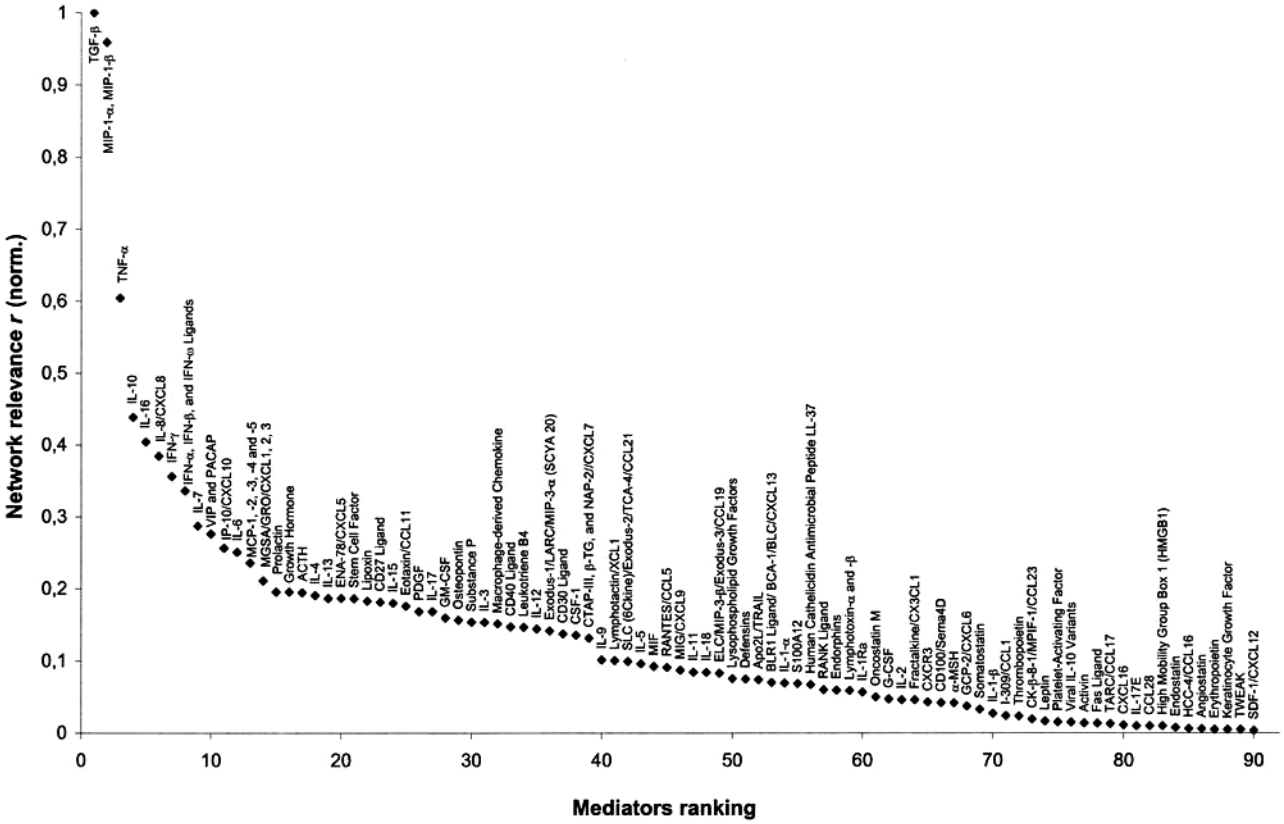

Fig. 3. Network relevance r of the mediators of the immune cell network. Mediators are ranked on the basis of their r value, where values are normalized so that the maximum value is set to 1. The 14 mediators in the highest ranks, i.e. those that play a crucial role for the network efficient communication, are all pro- or anti-inflammatory mediators, except IL-7 (a T-cell growth factor) and VIP/PACAP (an immuno-neuroendocrine mediator).

which have been experimentally studied and found to play a major role in aging and/or longevity, such as TGF-β (Forsey *et al*., 2003), TNF-α (Bruunsgaard *et al*., 2000), IL-10 (Lio *et al*., 2002a), IFN-γ (Lio *et al*., 2002b), IL-6 (Bonafè *et al*., 2001) and MCP-1 (Gerli *et al*., 2000), are included in the first two groups of relevant mediators, while two inflammatory cytokines, such as IL-1α and IL-1β, which do not seem to play a relevant role in these phenomena (Cavallone *et al*., 2003), appear in the group of lower rank mediators. Thus, mediators involved in innate immunity—the most ancestral branch of the IS—and in highly conserved defence pathways such as inflammation appear to provide a substantial contribution to the efficient communication of the IS network. Finally, the presence of a neuroendocrine hormone, ranking high in the list of immune network mediators, suggests that further investigations with a set of cells and mediators including those from the neural and the endocrine systems can shed light on the likely close interplay among these three systems (Ottaviani and Franceschi, 1996).

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