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Global analysis of the immune response

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ABSTRACT

The immune system may be seen as a complex system, characterized using tools developed in the study of such systems, for example, surface roughness and its associated Hurst exponent. We analyze densitometric (Panama blot) profiles of immune reactivity, to classify individuals into groups with similar roughness statistics. We focus on a population of individuals living in a region in which malaria endemic, as well as a control group from a disease-free region. Our analysis groups individuals according to the presence, or absence, of malaria symptoms and number of malaria manifestations. Applied to the Panama blot data, our method proves more effective at discriminating between groups than principal-components analysis or super-paramagnetic clustering. Our findings provide evidence that some phenomena observed in the immune system can be only understood from a global point of view. We observe similar tendencies between experimental immune profiles and those of artificial profiles, obtained from an immune network model. The statistical entropy of the experimental profiles is found to exhibit variations similar to those observed in the Hurst exponent.

1. Introduction

In recent decades, theories dealing with the complexity of the immune system have been proposed [1,2]. The focus in these works is on modeling the immune response. Despite the remarkable specificity of immune receptors to their targets, there is much evidence that the behavior of the immune system is not restricted to one-to-one antigen-antibody responses. Collective immune phenomena, such as tolerance, autoimmune diseases, and memory, among others, are far from well understood [3–9]

The immune system is formed of a huge number of components, with diverse interactions between them. Networks of interacting elements can be used to model some features of its dynamics. In these models, the elements (representing, for example, lymphocytes), interact via a set of well defined rules. It is important to note that most of the activity of the system is elicited not by foreign agents, but by self agents, i.e., molecules native to the organism.

The immune system may be seen as a complex system, that is, one composed of a large number of elements, with specific interactions. The interactions are often simple, though this need not always be the case. The dynamics of the system as a whole exhibit features (“emergent properties”) not found in the behavior of each element. Emergent properties and self-organized behavior are common characteristics of complex systems.

Many complex systems may be characterized by trajectories or, in some cases, fractal, n-dimensional surfaces in a given space; a key global property of such a surface is its roughness statistics [10,11]. One hopes, by analyzing the roughness, to classify different regimes of behavior. Analysis of surfaces generated by apparently unrelated processes, may also help to identify underlying similarities in their governing equations or dynamical rules [12–14].

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To apply this approach to global properties of the immune response, one requires an overall picture of the immune repertoire. We do this by studying a large set of reactivities against another (large) set of antigens, derived from tissue extracts. The experimental technique generating information on the set of reactivities is called *immunobloting*. The results of this technique is usually analyzed through multiparametric statistical analysis.

One of the problems in describing the immune system as a multiconnected network, is how to deal with its global connectivity. A bold attempt in this direction was made by the inventors of the Panama blot assay [15,16]. A modified immunoblot assay, the Panama blot, is not aimed at the detection or quantification of a specific antigen-antibody reaction. Rather, it permits comparison of patterns of global reactivity of natural serum immunoglobulins with complex antigenic mixtures. Antigenic extracts consisting of whole organs (brain, liver, muscle, etc.) or whole bacterial cultures (such as Escherichia coli), have been used to generate these mixtures. The reaction of serum immunoglobulins with these mixtures results in activity profiles, which are used to compare patterns of different individuals, or the history of a given individual, rather than to detect or measure individual immunological reactions, as is customary in serology.

Traditionally, those profiles are described by points in a n-dimensional space, where the set of coordinates corresponds to the rescaled peaks of each profile. The dimensionality of the space is then reduced, using principal component analysis (PCA), and clusters of similar individuals are identified via statistical analysis. In the present work, we introduce an approach to study the densitometric profiles, and arrange individuals in groups, based on roughness statistics. We focus on a population of individuals from a region in which malaria is endemic. The individuals of this population can be classified into several groups, according to the presence or absence of malaria symptoms, number of malarial manifestations, etc. A control population, from a malaria-free region, is used to define reference characteristics of the reactivity profiles.

In our approach, the densitometric profile is represented as a function $h(x)$, where the variable x represents position on the gel (a function of protein molecular weight), and h reflects the reactivity at this position. The functions $h(x)$ corresponding to each individual are then analyzed, using several techniques: PCA, superparamagnetic clustering, and analysis of roughness, leading to calculation of the Hurst exponent of the profile. We show that the Hurst exponent, calculated from single parametric statistical analysis, separates individuals from malaria-endemic and malaria-free regions. We compare our method with the traditional one and with a clustering technique introduced by Domany et al [17], based on phase transitions in a Potts model, with many states and distance-dependent interactions. Our method shows better discrimination than the other two studied. More importantly, the success of profile-based methods highlights the utility of global analysis of the immune system, and suggests the presence of collective phenomena in this system.

2. Materials and methods

Immune reactivity profiling, via the Panama Blot method, was performed on blood collected from a set of 78 individuals having similar lifestyles, living in a region of the State of Mato Grosso, Brazil, in which malaria is endemic. Individuals living in this region are continuously exposed to the malaria parasite. For comparison, blood from a group of 10 individuals residing in Belo Horizonte (State of Minas Gerais, Brazil), a region in which malaria is not endemic, was also analyzed. The individuals in the endemic region are classified into groups A–F, according to their history of malaria exposure:

A: those whose blood shows no evidence of the parasite;
B: those that have presented symptoms of malaria, between 1 to 10 times, but were asymptomatic at the time of blood collection;
C: those that have presented symptoms more than 10 times, but were asymptomatic at the time of blood collection;
D: those symptomatic at the time of blood collection;
E: those with the parasite in their blood, but asymptomatic for the disease at the time of collection.

The individuals from the non-endemic region are characterized as:

F: those never having been exposed to the parasite.

Details about the experimental procedures can be found in Ref. [18].

2.1. Experiment: Panama Blot

Immune profiles were obtained through reactions between a population of immunoglobulins, and an extract containing a large number of proteins or peptides (protein fragments). In the Panama Blot method, the protein extract is generally prepared from a cell culture or tissue; in the present case it was prepared from human liver or brain (from cadavers). The extract is dissolved in a pH-adjusted solution, so that the proteins acquire electric charge, necessary for electrophoresis [19]. The solution is poured over a polyacrylamide gel confined between glass plates, and subject to a constant electric field in the plane of the plates. The proteins then migrate within the gel, with velocities depending on their molecular weight and charge.

Once the extract proteins have been separated via electrophoresis, they are transferred, by application of an electric field perpendicular to the plates, to a nitrocellulose membrane, for subsequent reaction with serum (Fig. 1). The membrane, with the proteins adsorbed on its surface, is placed on a mold bearing a series of narrow, parallel channels. The channels are then loaded with serum samples, each from a different individual, and allowed to react with the extract proteins for a period of four hours.