CHAPTER 15

THE NATURAL AUTOANTIBODY REPERTOIRE IN NEWBORNS AND ADULTS: A Current Overview

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Abstract: Antibody networks have been studied in the past based on the connectivity between idiotypes and anti-idiotypes—antibodies that bind one another. Here we call attention to a different network of antibodies, antibodies connected by their reactivities to sets of antigens—the antigen-reactivity network. The recent development of antigen microarray chip technology for detecting global patterns of antibody reactivities makes it possible to study the immune system quantitatively using network analysis tools. Here, we review the analyses of IgM and IgG autoantibody reactivities of sera of mothers and their offspring (umbilical cords) to 300 defined self-antigens; the autoantibody reactivities present in cord blood represent the natural autoimmune repertories with which healthy humans begin life and the mothers' reactivities reflect the development of the repertoires in healthy young adults. Comparing the cord and maternal reactivities using several analytic tools led to the following conclusions: (1) The IgG repertoires showed a high correlation between each mother and her newborn; the IgM repertoires of all the cords were very similar and each cord differed from its mother's IgM repertoire. Thus, different humans are born with very similar IgM autoantibodies produced in utero and with unique IgG autoantibodies found in their individual mothers. (2) Autoantibody repertoires appear to be structured into sets of reactivities that are organized into cliques—reactivities to particular antigens are correlated. (3) Autoantibody repertoires are organized as networks of reactivities in which certain key antigen reactivities dominate the network—the dominant antigen reactivities manifest a "causal" relationship to sets of other correlated reactivities. Thus, repertoires of autoantibodies in healthy subjects, the immunological homunculus, are structured in hierarchies of antigen reactivities.

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INTRODUCTION

The immune system is a key player in daily body maintenance and defense and its proper functionality is vital both to the survival of the individual and to its well-being. The immune system is composed of complex networks of molecules, cells and organs that act together to maintain and repair the body and protect it.[1-6] The immune system is dynamic, a constantly evolving network whose complexity is comparable to that of the central nervous system.

Every exposure to an antigen, be it an invader (bacteria, virus) or a self component alters the immune state and the antibody repertoire. Antibodies binding to molecules of the body itself—autoantibodies—are associated with the pathologic inflammatory processes that cause autoimmune diseases. However, autoantibodies in healthy individuals, in contrast to pathogenic autoantibodies, are thought to function in body maintenance and healing. It appears that naturally occurring autoantibodies (NAbs) and auto-reactive T cells in healthy individuals are directed to a selected, limited set of self-molecules. The autoimmune repertoire serves the immune system as an internal representation of the body, and has been termed the immunological homunculus.[1,2] Natural autoimmune T cells and B cells and autoantibodies may provide an early immune response to pathogens, expressing molecules that are cross-reactive with particular self-antigens. An example is the response to bacterial heat shock proteins and to other molecules that are highly conserved. Natural autoimmunity has also been proposed to prevent pathogenic autoimmunity by generating regulatory circuits or by blocking access by potentially pathogenic agents to key self-antigens.[7]

To characterize the immunological homunculus network (IHN), we used informatics tools to study patterns of antibody reactivity to hundreds of self-molecules (of our design) arrayed on glass slides—an antigen chip.[8-11] This immune microarray (Fig. 1) consists of various antigens covalently linked to the surface of a glass slide. A drop of blood serum (or any other body fluid) is tested for antibody reactivity by measuring antibody binding to each antigen spot using fluorescence labeling. Note that the binding of antibodies to a spotted antigen cannot tell us about the stimulus that induced the antibodies, and it cannot define the affinity or the specificity of any particular antibody or collective of antibodies. Indeed, a positive antigen-binding signal probably reflects a polyclonal mixture of antibodies binding to a variety of structural epitopes exposed by each spotted antigen.

Results using the antigen chip suggest that the particular self-reactivities comprising the IHN could serve as a set of biomarkers that help the immune system to initiate and regulate the inflammatory processes that maintain the body.[7] A full description of the immunological homunculus network would require information about an individual's T-cell antigen specificities (repertoire) and frequencies of T-cell functional types (Th1, Th2, Th3, CTL, Treg and so forth), their B-cell repertoire and B-cell types, autoantibody repertoire and antibody isotypes, and innate immune cells (macrophages, dendritic cells, neutrophils and so forth). Most of this information is not accessible—in fact, much of it is not characterized in detail but known only in general terms. Nevertheless, antibodies are precisely measurable and the pattern of one's global repertoire of autoantibodies in blood and body fluids is accessible. The autoantibody homunculus, at least, can be consulted. Moreover, microarray technology combined with advanced system-level analysis methods has opened new opportunities for approaching the vast information stored in antibody repertoires. Therefore, it has been proposed that the global pattern of autoantibodies can reveal various states of the immune network and provide some insights about the body state of the individual.[7,10]

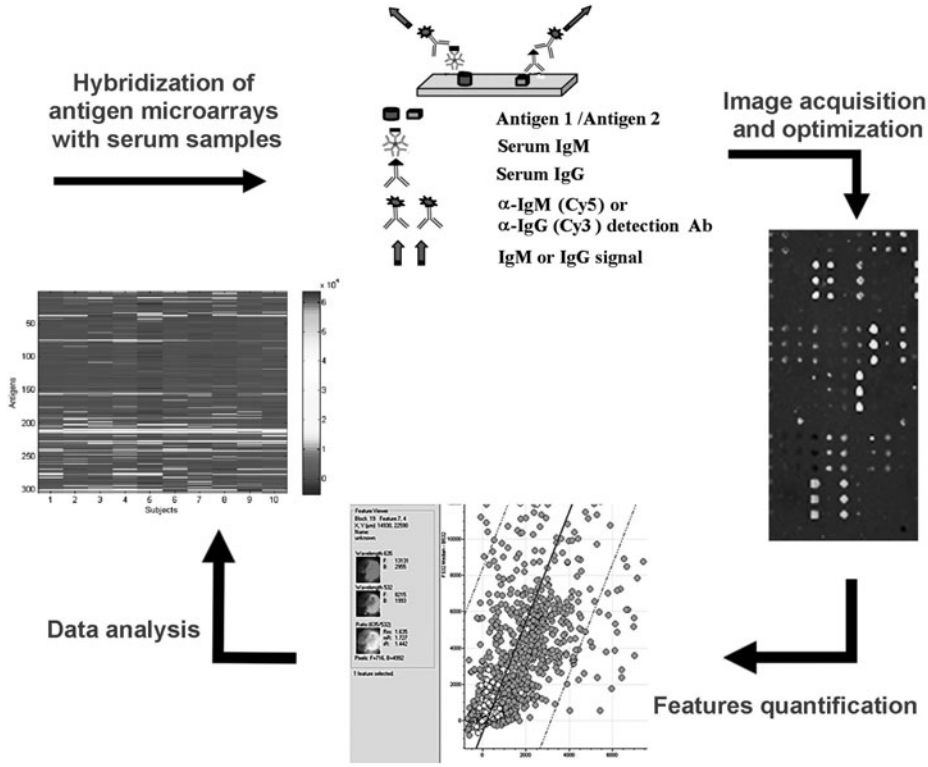

Figure 1. Schematic flow of the antigen chip from production to analysis. Step 1—Hybridization of antigen microarrays with serum samples: The tested body fluid (serum in this case) is hybridized on the antigen microarray to react with the chosen antigens. Then, secondary antibodies labeled with fluorescent dyes (Cy3 and Cy5) are added to assess the amount of antibody reactivity to each antigen. Step 2—Image acquisition and optimization: After incubating, washing and drying, the microarray is ready for reading and the fluorescence intensities are scanned. Step 3—Feature quantification: The scanned fluorescent images are translated to quantitative antibody reactivities based on the spot fluorescence level. The process includes optimization of the background reading and removal of problematic spots. Step 4—Data analysis: This step includes illuminating information hidden in the data using appropriate algorithms, to be explained in the text.

THE MATERNAL AND NEWBORN AUTOANTIBODY REPERTOIRES

The immune system expresses both the genetic endowment of the individual and the life experience of the individual. During pregnancy, the immune system has a very special role in the mother–offspring dyad by providing not only defense against infectious agents, but also IgG antibodies of the mother, which are actively transferred to the fetus. Immediately after birth, the immune system deals with post-natal adaptation to life in a continuous and dynamic process. Like the central nervous system, the immune system is *self-organizing*: it begins with genetically coded, primary instructions, to which it adds information retrieved from the individual's experience with the environment in

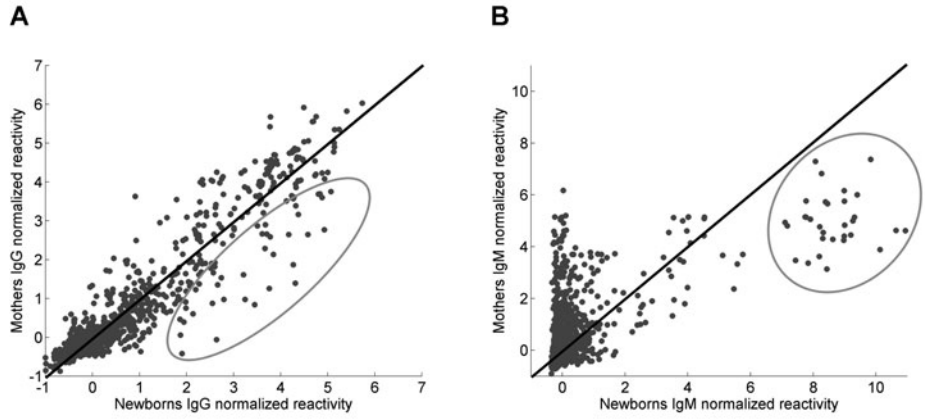

Figure 2. The normalized antibody reactivities of newborns (x axis) and mothers (y axis) as scatter plots. For the IgG scatter plot (A), most of the antigens exhibit similar reactivities of newborns and mothers, where only a small fraction of the antigens shows significant deviations from this behavior. We note the relatively small but significant group of antigens marked by an ellipse indicating antigens with higher reactivities in the newborns. For the IgM scatter plot (B), 2 types of antigen populations can be detected, the vast majority of antigens are characterized by relatively low reactivities of the newborns compared with their mothers, but a second group (marked by an ellipse) is characterized by high levels of reactivities, where the newborns' reactivities are somewhat higher than the mothers'.

health and disease. Just as each person develops a unique brain, each person develops an individualized immune system.[2]

To characterize the development of natural autoantibodies from birth to adulthood, we re-examined the antibody binding of 10 pairs of mothers (blood sample) and their newborns (umbilical cord sample) to 300 (mostly self) antigens previously reported by Merbl et al.[12] Figure 2 depicts the normalized antibody reactivities of the newborns (x axis) and their mothers (y axis) as scatter plots, such that each point in the graph represents a specific antigen reactivity in the maternal-newborn plan.

For the IgG isotype (Fig. 2A), we observed basically similar reactivity levels of the mothers and their newborns, reflected by the fact that the points form approximately a symmetric distribution along the diagonal. This can be explained by the fact that most of the fetal IgG antibodies originate from the mother, actively transported across the placenta.[13] Note, however, that there is a relatively small but significant group of antigen reactivities marked by the gray ellipse indicating higher reactivities in the newborns. Several factors might explain this phenomenon: selective transfer of certain IgG antibodies, the accumulation of such antibodies in the fetus, dissociation of maternal IgG-IgM antibody complexes during active transfer across the placenta and active production by the fetus of IgG antibodies to these antigens. Indeed, it has been suggested by Akilesh et al.[14] that, in addition to the role of the placental FcR (FcRn) in transferring maternal IgG to the fetus, the placenta also protects bound IgG from catabolism and maintains high IgG serum levels. Therefore, an accumulation of certain IgG antibodies could take place if these antibodies are transferred more than others and the rate of antibody catabolism differs between the fetus and the mother.

ANTIGEN-REACTIVITY CORRELATIONS

For many autoantibodies of the IgM isotype (Fig. 2B), the levels of the mothers are generally higher than those of their newborns; but certain antibody-reactivities manifest higher reactivity in the newborns. In contrast to IgG, IgM antibodies do not cross the placenta, so IgM autoantibodies in cord blood must have been produced by the developing fetus before birth.[12,15] Therefore, it is not surprising that some fetal reactivities differ from those of the mother.

Recently, we extended the study reported by Merbl et al.[12] by a different analysis of additional data from five females, who were not pregnant, and from eight newborns at birth and at day seven.[15] The analysis was performed from the perspective of the immune system as a complex functional network of antibodies by correlating their reactivities among themselves. We analyzed the correlations of antigen reactivities and of subjects to detect relationships between particular antigen-reactivities in the populations of mothers and newborns in both their IgG and IgM repertoires (Fig. 3).

The IgG subject correlation matrix in Figure 3A depicts a high correlation (light gray or white) between each mother and her offspring (red squares), again, indicating the maternal source of the IgG fetal repertoire. The high correlation values between each newborn at day 1 and again at day 7 (green squares) indicate conservation of the IgG autoantibody repertoire. Note that there are low correlation values between each of the adults and each of the infants and between the groups, indicating the individual nature of the IgG repertoire.

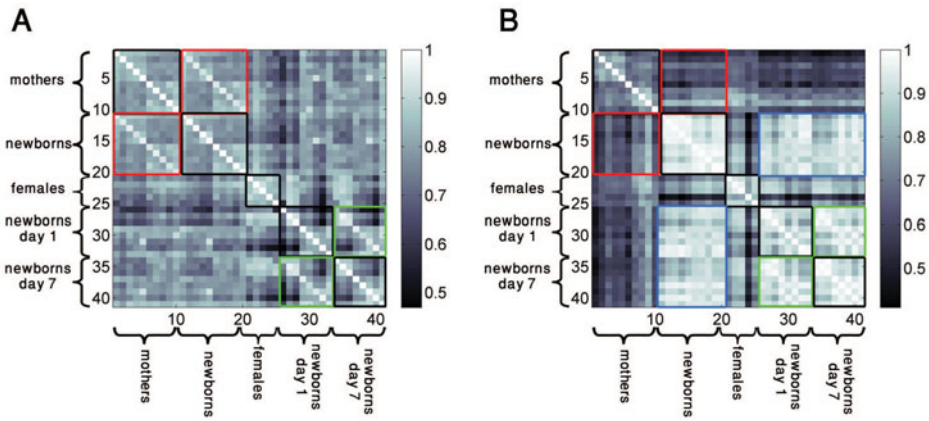

Figure 3. The subject correlation matrices for the IgG (A) and IgM (B) isotypes, color coded from low correlation (dark gray) to high correlation (white). The matrices are ordered according to the mothers (1–10), and their newborns (11–20), five females (21–25) and the additional newborns at birth (26–33) and at day 7 (34–41). The white diagonal lines are formed by the absolute correlations between each subject in both the x and y axes. A) The IgG correlation matrix: note that there are additional white diagonals in the red squares; this signifies the high correlation between each mother and her newborn; the additional diagonals in the green squares signify the high correlation between each newborn at day 1 and day 7. B) The IgM correlation matrix: note the strong correlation between all the newborns (blue squares) and the very weak correlation between each mother and her newborn (red squares). The high correlation values inside the green squares indicate that the IgM autoantibody repertoires changed very little in the first 7 d of life. We are now studying whether antibodies to bacteria develop in the first few weeks after birth and the effect of bacterial colonization of the gut on the development of the autoantibody repertoire.

The IgM subject correlation matrix (Fig. 3B) shows very weak correlation values (dark gray and black) between each mother and her newborn, with strong correlations between all the newborns (blue square) indicating the universal (common) nature of the congenital IgM repertoire.

MODULAR ORGANIZATION AND ANTIGEN CLIQUES

The antigen correlation matrices described above were analyzed using the functional holography (FH) method of Baruchi et al.,[16] originally devised for analyzing recorded brain activity, and also shown to be useful in the analysis of gene-expression data. In a recent work, this method was used to identify expression relations between genes and gene network motifs.[17] By using this approach on the antigen reactivity data, we were able to unveil new information about functional relations between self-antigens—modular organization of the autoantibody network and the formation of self-antigen cliques.

The modular organization of IgM antibodies is shown in Figure 4 for the 10 mothers and added women (A) and for the newborns (B). It was found that the mothers and the added women exhibit modular organization of their IgM repertoires (Fig. 4A) into antigen cliques—distinct subgroups of highly correlated antigen reactivities. In contrast to the IgM of the mothers, the IgM repertoires of the newborns were not organized into separate antigen cliques (Fig. 4B). The presence of IgM cliques of reactivity in the mothers and their lack in the newborns suggest that humans develop coordinated sets of IgM autoantibody reactivities during healthy post-natal development. The IgG reactivities of the newborns are more organized than their IgM reactivities (data not shown), as expected from the transfer of maternal IgG to the fetus.

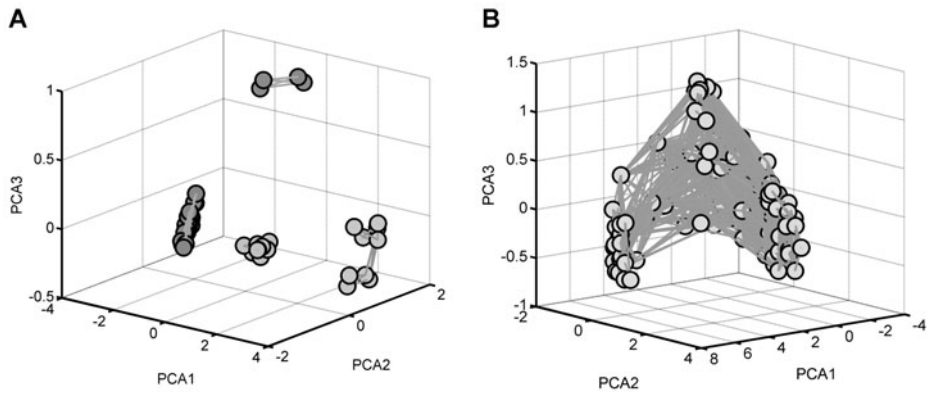

Figure 4. IgM antigen cliques. We present the antigen-reactivity correlation information in a 3-dimensional principal component analysis (PCA) space, whose axes are the three leading principal vectors computed by the PCA algorithm. Each antigen is placed in this space according to its three "eigenvalues" for the three leading principal vectors. Note that antigens that manifest high normalized correlations will be placed in close vicinity in the PCA space. A) The antigen network of the 45 antigens that compose the strong cliques in the maternal data set. B) The antigen network for the large cluster of 150 antigens identified for the newborns. In the presentation of the antigen networks, nodes (antigens) with high correlations (< 0.85) are shown linked by the orange lines. A color version of this image is available online at www.landesbioscience.com/curie.

IMMUNE NETWORK ARCHITECTURE AND IMMUNE TREES

Natural antibody networks have been studied in the past based on the connectivity between idiotypes and anti-idiotypes—antibodies that bind one another.[18-20] More recently, we extended our analysis of autoantibody reactivities[15] by applying graph and network theory analysis methods.[21-23] This approach calls attention to a different network of antibodies, autoantibodies associated by their reactivities to sets of self-antigens. In this immune network, the nodes (circles) represent the antigen reactivities and the links between the nodes (often called edges) represent the relationships between the autoantibody reactivities calculated for each group of subjects. In other words, an immune network for a given group of subjects corresponds to the network of similarities between antigen reactivities within that group of subjects.

In the complete network of antigen correlations every node is linked to all other nodes. However, most of the links are not significant as they correspond to very weak correlations. Therefore, the complete graph contains a high level of non-significant information that may mask the essential motifs. To extract the relevant information, it is possible to generate a condensed representation of the complete network by using various methods such as the Minimal Spanning Tree (MST) methodology,[24-27] which we employed here. The MST is a widely used sub-graph of the complete network which is constructed using a special algorithm that enables us to extract the most relevant information from the full network.[28] The idea of the MST algorithm is to select the subset of more informative links (about the hierarchical structure of the system) and reduce the complete all-to-all network (that contains $N(N\text{-}1)$ links) to a representative sub-graph (that contains only $N\text{-}1$ links). Hence, generating the maximum information immune networks (or immune trees) by the MST, makes it possible to investigate essential organizational motifs, such as the network topological organization. Moreover, similar to neuronal and gene networks, the immune system can exhibit activated and inhibited reactivities, such that both positive and negative antigen correlations contain important information. While the typical construction of the correlation-based MST has a bias toward strong positive correlations, we analyzed here the absolute value of the antigen-antigen correlations, in order to give equal importance to both strong positive and negative correlations.[29] We thus constructed the immune MST for each group of subjects (mothers/newborns), each isotype (IgG/IgM) and integrated isotype tree for each group[29] (Fig. 5).

We assessed and compared the topological organization of the IgG and IgM immune trees of the mothers and newborns. Next, the networks of the two subject groups, mothers and newborns, were compared by employing the widely used divergence rate measure.[30] The analysis revealed a high topological similarity between the newborns' and mothers' IgG networks and significant topological differences between the newborns' and the maternal IgM networks. These results indicate partial conservation of the IgG immune network topology from birth to adulthood, and significant reorganization of the IgM immune networks during the healthy development of the immune system, also shown above using other types of analysis.

Our previous work[29] uncovered previously unrecognized features of natural autoantibody networks. It was shown that the repertoires of mother and newborn manifest generally different network architectures: the composite tree of IgG and IgM reactivities shows that the nodes of IgG and IgM reactivities are largely overlapping in the mothers, but are mostly distinct in the newborns; it is quite possible that the overlap between the isotypes in the mothers results from adaptive immune responses to particular antigens.

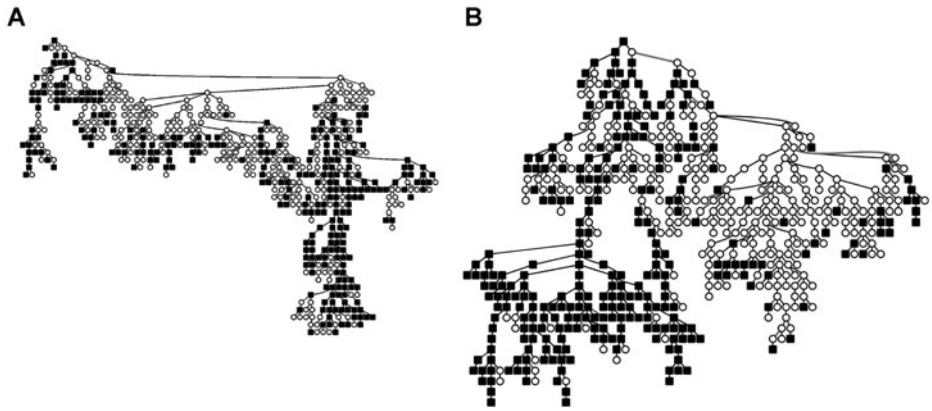

Figure 5. Hierarchical organization of the "integrated similarity immune trees" for the IgM and IgG isotypes. A) The Minimum Spanning Tree (MST) of the maternal data set, and B) the MST of the newborns. The black squares and white circles (nodes) represent the IgG and IgM isotypes respectively. We present the negative correlations between two nodes by the use of red or black lines, reflecting the fact that the organization of these immune networks can exhibit activated and inhibited reactivity responses. Note that many clusters or sub-trees are composed of a single isotype in the newborns, but less so for the mothers, where the sub-trees of IgG and IgM appear to overlap to a great extent.

Both negative and positive relations between antigen reactivities participate in connectivity throughout the MST network, reflecting the fact that the organization of these immune networks can exhibit activated as well as inhibited reactivity response.

This methodology is unique in its description of the network-tree architecture of the natural autoantibody repertoires in healthy mothers and newborns; the causal mechanisms responsible for this network architecture and for the differences between mothers and newborns need to be investigated.

ANTIGEN DEPENDENCY NETWORKS AND INFLUENTIAL ANTIGEN-REACTIVITIES

In this section we introduce a system-level analysis of antigen-dependency networks as a step toward the inference of causal relations between antigens.[31] The analysis is based on the measure of "partial correlations," which are becoming ever more widely used to investigate complex systems. Examples range from studies of biological systems such as gene networks[30,32] to financial systems.[33,34]

In simple words, the partial (or residual) correlation is a measure of the effect (or contribution) of a given antigen-reactivity, say *j*, on the correlations between another pair of antigen-reactivities, say *i* and *k*. This partial correlation approach enables one to define the hypothetical influence of antigen-reactivity *j*, as the sum of the influence of that antigen-reactivity (*j)* on all other antigen-reactivities *i*.[31] In this construction of "antigen dependency networks," the nodes represent the antigens spotted on the chip and the arrows between the nodes indicate the directionality of the influence—which antigen-reactivity influences which other antigen-reactivities; the approach defines a kind of causal influence of one reactivity on other reactivities. It's as if immunization to

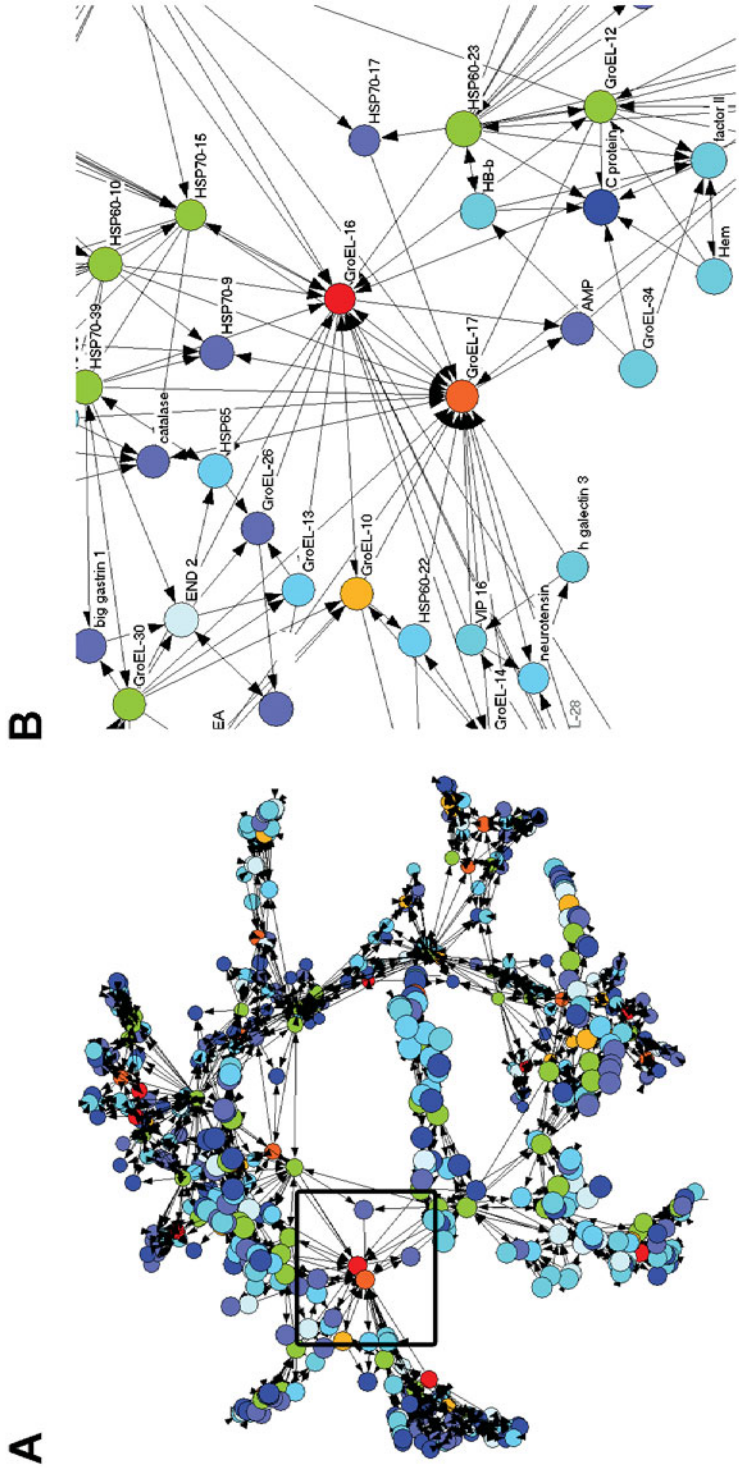

Figure 6. Figure legend on following page.

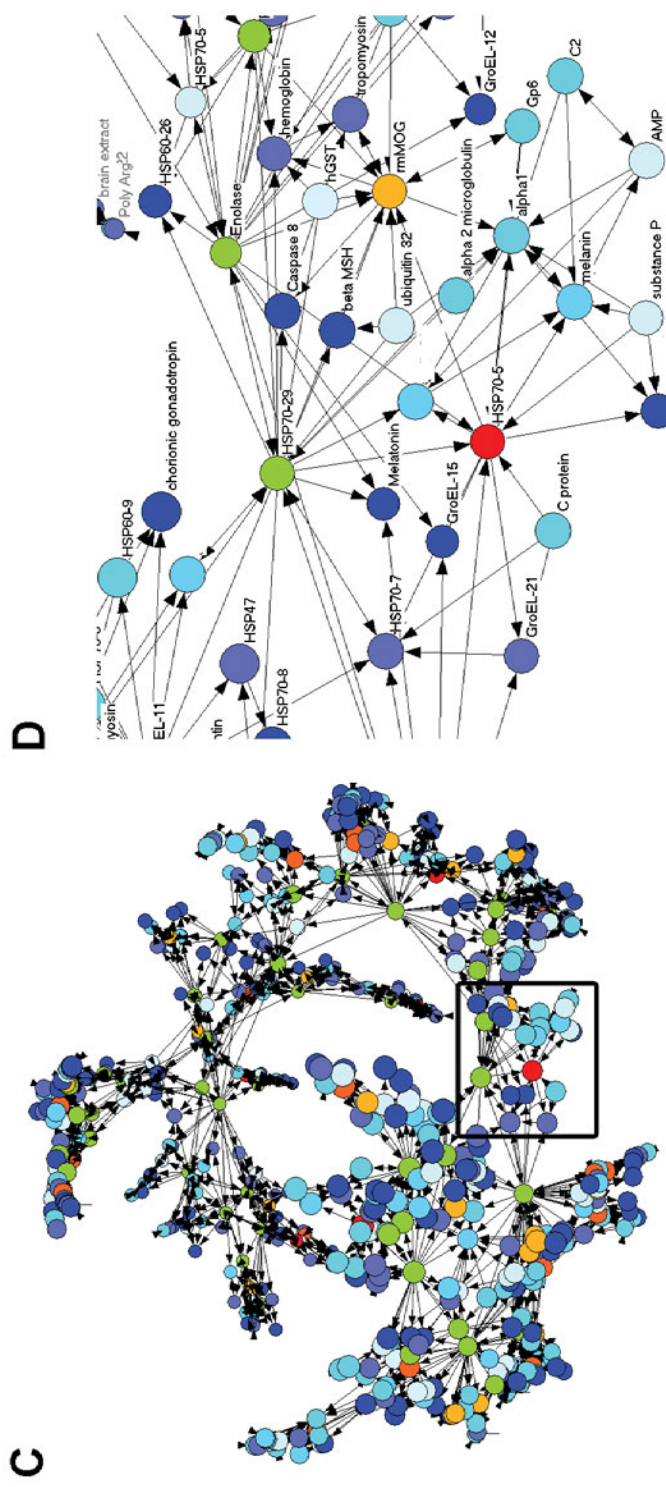

Figure 6, continued from previous page. A hybrid presentation of the dependency network for the combined IgG and IgM isotypes. The selected layout, Fruchterman-Reingold 3D, visualizes the differences between the maternal (A) and the newborn (C) networks. The black squares indicate the area that was zoomed in for A in B and for C in D. The colors indicate the strength of the system level influence (SLI) of each antigen on the correlations between all other antigen pairs, ranging from the most affecting antigens (dark red) to the least affecting antigens (dark blue). The arrows indicate the directionality of the influence, in other words, which is the antigen that positively affects the correlations of which other antigen. Note the wide dispersal of highly affecting antigens in the networks.

one particular antigen induces an immune reaction to other antigens. The colors indicate the strength of the system-level influence (SLI) of each antigen on the correlations between all other antigen pairs, from the most affecting antigens (dark red) to the least affecting antigen (dark blue) in the network. In other words, a dependency network for a given group of subjects corresponds to the network of dependencies between antigen reactivities within that group of subjects. A concrete example can be seen in the case where autoimmunization to one myelin antigen epitope in multiple sclerosis patients might lead to "spreading" to other antigen reactivities in the course of myelin damage.[35] Influential antigen-reactivities act as "drivers" for additional reactivities to form a network.

We constructed the "antigen dependency networks" for the groups of mothers and newborns.[31] We first constructed the IgG, IgM and combined IgG and IgM "networks of antigen dependencies" for the two groups (Fig. 6). Next, the networks of the two subject groups were compared by employing two technical measures that were developed in the context of network theory and are widely used—the divergence rate[30] and modularity score.[4] The first method was used to compare the position of each antigen-reactivity and its connections. The second method was used to assess the differences in the modular organization between the two networks—maternal and newborn. We found a higher modularity for the maternal IgG network. We found that the most influential "driver" antigens in the mothers and newborns are composed of both IgG and IgM isotypes, which points to a role for the maternally transferred IgG in influencing the newborn's network. Thus, the analysis of antigen dependency networks enabled us to unveil driver autoantibody reactivities in the maturation of the immune system. Which are these driver self-antigens?

The analysis of the system-level influence (SLI) revealed that about 10–15% of the antigen reactivities are drivers (colored in red and orange in Fig. 6); these antigen-reactivities manifest significantly higher driver influences than the other antigen reactivities. These driver reactivities tend to be evenly spread in the dependency networks. We found that the driver antigen reactivities of the IgG networks are prominently composed of epitopes of Heat Shock Proteins (HSPs) (~50% of the top 20 most influential antigens (antigens with high SLI scores) are HSP60 related and ~20% are HSP70 related). It is true that the array of antigens spotted on the chip contains many HSP peptide epitopes (~30% of all the spots). However, the enrichment of highly-ranked HSP molecules in the IgG networks and the absence of HSP epitopes as drivers in the IgM networks suggest that the dominance of these HSP eptiopes for the IgG repertoires is not merely an artifact. Note that HSP60 appears to function as a biomarker of inflammation and stress for the immune system,[7,36] which suits the position of HSPs as drivers in the maternal and newborn networks.

DISCUSSION

The results presented here support the concept of the immunological homunculus[1,37-39]—the idea that the healthy immune system includes autoreactivity to a selected set of particular self-molecules. Indeed, the homunculus idea triggered the development of our antigen microarray chip.[10] The fact that the primary autoantibody homunculus arises during the uterine life of the fetus, an environment normally free of foreign antigens, suggests that self-molecules are likely to serve as the immunogenic stimulus inducing IgM and IgA autoantibodies. The exact mechanism of this stimulus is still unknown, but it seems that the development of B cells, like that of T cells, may involve positive selection for self-reactivity.[40]

As we have shown here, the B-cell arm of the immune system has evolved to produce IgM autoantibodies to certain self-molecules even before birth; hence it is reasonable to conclude that these autoantibodies may provide potential advantages that offset the occasional autoimmune disease in post-natal life associated with their target autoreactivities.[1,41] It is conceivable that NAbs, in recognizing specific body molecules, help the immune system gather and integrate essential information about the state of the body and thus provide potential health benefits.[2] In any case, the inclusion of some major disease–associated self antigens in the innate autoantibody repertoire suggests that autoimmune disease could arise through a lapse in the regulation of natural, otherwise benign, autoimmunity.[42]

For the IgM autoantibodies, which are not transferred from mother to fetus, we found that all the newborns shared a universal innate immune profile, in agreement with the concept of the immunological homunculus—the immune system's internal image of key body molecules.[42] On the other hand, the maternal IgM and IgG autoantibody repertoires are highly diverse, implying that healthy development of the autoimmune state from birth to adulthood arises from immunological learning according to one's personal life experience. In other words, it seems that the immunological homunculus is not static but responds with personal immune experience. At present, we do not know whether a healthy maturation of the autoantibody repertoire is induced by immune experience with foreign antigens cross-reactive with self and/or by autoimmune contact with sets of self-antigens during normal body maintenance.[2,3] Study of the autoantibody repertoires developing in germ-free and antigen-free animals would shed light on this question. Moreover, it would be important to study whether particular types of autoimmune repertoire organization are potential seeds of the later development of clinical autoimmune disease.[9,10]

Analyzing the maternal IgM and IgG autoantibody correlations, we found that immune state diversity goes hand in hand with the development of a modular organization, reflected by the formation of antigen reactivity cliques or functional immune groups. The dominance of HSP epitopes[43] as "drivers" of other autoantibody reactivities is another indication of the intrinsic organization of natural autoimmunity.

The observation of an organized network of autoantibodies in mothers and newborns challenges the Clonal-Selection Theory (CST) of adaptive immunity, which has dominated immunological thinking for the past half-century.[44] The classical CST[2,45] discourse emphasized the functional independence of individual immune cells and so a network of antibody reactivities was outside of CST expectations. Moreover, according to the CST, it was inconceivable that the healthy immune system recognizes components of the body, since any recognition of self-molecules was thought to produce an autoimmune disease. Thus, the CST postulates that the immune repertoire, during development, must be purged of lymphocytes bearing receptors that could bind self-molecules. The self, as viewed by the CST, goes unnoticed and autoimmunity is forbidden. The present results do not fit the worldview of the CST. The present findings indicate that the healthy immune system, even before birth as the self-reactive IgM in the umbilical cord illustrates, recognizes self-molecules and, moreover, does so in a highly ordered architecture of reactivities. However, larger data sets are needed to verify the existence of autoantibody cliques and the complete identification of the antigen reactivities of each clique.

In general, the results presented here are consistent with the concept of the Immunological Homunculus, the idea that healthy immune repertoires contain certain T cells and B cells that have been positively selected to respond to key body molecules to form a functional "internal image" of the body.[2,3,7,36] This homunculus theory is

based on the regularity of immune self-recognition, consistently observed in healthy individuals. In practice, autoreactivity is not the aberration proposed by the CST, but is actually structured within the functional architecture of the immune system. The dominant position of particular antigen-reactivities, such as HSP epitopes would seem to reflect the biases of selected targets of self-recognition.[32] Note that both the CST and the anti-idiotypic network paradigms are based on individual differences between the immune repertoires developed by individual subjects; the immunological homunculus idea, in contrast, highlights the existence of antigen reactivities shared by individuals within a population. The relative uniformity of IgM autoantibody repertoires in newborns as a group[12,15] fits the homunculus idea: The demonstration of antigen dependency networks with dominant driver reactivities provides an additional way to view the homunculus.

The prevalence of dominant NAbs to body molecules, such as HSPs, suggests that such autoantibodies might provide some advantage to the organism. In fact, it has been suggested that NAbs might function to prevent autoimmune disease[46] or serve as immune biomarkers of the body state.[7,36] The persistence of dominant driver reactivities from newborns to mothers might account for the reports that IgM repertoires show little change from birth.[47-49] These other studies[49-51] were done using crude tissue blots of undefined self-molecules, while the antigen microarray technology used here apparently made it possible to detect the changes, despite the persisting drivers, in fine specificity of the autoimmune repertoire occurring subsequent to birth.

At present, we can view the newborn and maternal repertoires as snapshots of the dynamic evolution of the autoantibody repertoire during healthy maturation. It is reasonable to consider that the mothers at their own births arrived outfitted with the common IgM autoantibody repertoire we found shared by all the newborns.[12,15] Thus, we might reason that the state of the maternal repertoire probably reflects its physiological evolution from the newborn state. To test this hypothesis, we are presently undertaking a longitudinal study of the evolution of the antibody repertoires of individual humans from birth to an array of antigens including both self and foreign molecules.

Niels Jerne proposed that, in addition to the recognition of foreign antigens, lymphocytes and antibodies also respond to the unique antigen receptors—idiotopes—of other lymphocytes.[19,20] The interactions between idiotopes create an anti-idiotypic regulatory network. The Jerne idiotypic network theory views the immune system as a dynamical network of continuously interacting cells and antibodies,[50-52] even in the absence of external antigens.[53] Hence, in contrast to the CST concept of clonal independence, Jerne proposed that the immune response to a foreign antigen takes place when the entry of an antigen into the system perturbs the internal homeostasis of interacting idiotopes.[19,20,54] The organized autoantigen-reactivity networks expressed at the level of the newborn population, however, adds a level of organization to the concept of the individualized anti-idiotypic networks proposed by Jerne.

CONCLUSION

The discovery and analysis of immune system network architecture shown here and elsewhere[55] serve as an introduction to basic questions in systems immunology: What mechanisms are responsible for driver antigen-reactivities; what is the dynamic function of the relatively large number of most influential antigens that serve as drivers;

and how is the architecture of the immune network modified by vaccinations, infections, neoplasia, autoimmune diseases and other perturbations of immune homeostasis? The antigen microarray provides one tool to study these questions.

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