Detection of Complement Activation on Antigen Microarrays Generates Functional Antibody Profiles and Helps Characterization of Disease-Associated Changes of the Antibody Repertoire[1]

Krisztián Papp,* Péter Végh,† Kata Miklós,[2‡] Julianna Németh,[2‡] Klára Rásky,§ Ferenc Péterfy,§ Anna Erdei,*† and József Prechl[3]*

Humoral immune responses are traditionally characterized by determining the presence and quality of Abs specific for certain Ags. Arraying of large numbers of Ags allows the parallel measurement of Abs, generating patterns called Ab profiles. Functional characterization of these Abs could help draw an even more informative map of an immune response. To generate functional Ab profiles we simultaneously tested not only IgM, IgG, and IgA binding to, but also complement activation by, a panel of endogenous and exogenous Ags printed as microarrays, using normal and autoimmune human sera. We show that complement activation by a particular Ag in a given individual cannot be predicted by the measurement of Ag-specific Abs, despite a general correlation between the amount of Ag-bound Ab and the deposited C3 fragments. This is due to both differences in the isotypes that dominate in the recognition of an Ag and individual variations for a given isotype, resulting in altered complement activation potential. Thus, Ag-specific C3 deposition can be used as an additional parameter in immune response monitoring. This is exemplified by comparing the coordinates of Ags, used for the diagnosis of systemic lupus erythematosus, of normal and autoimmune serum samples in a two-dimensional space derived from C3 deposition and Ab binding. Since cleavage fragments of C3 mediate important immunological processes, we propose that measurement of their deposition on Ag microarrays, in addition to Ab profiling, can provide useful functional signature about the tested serum. ***The Journal of Immunology,*** **2008, 181: 8162–8169.**

Effector functions of Abs are mediated primarily via receptors for the Fc region and the initiation of the complement cascade. Abs belonging to different classes, subclasses, glycoforms, and having different affinities possess highly variable effector potentials. Protectivity or pathogenicity against defined targets therefore depends on these characteristics of the polyclonal response of an individual.

Ag arrays are becoming the tools of choice for generating profiles of targets of the humoral immune response. Ag collections can be proteomic scale protein sets (1–3) or arrays focusing on certain disease-specific Ags (4–6). These profiling approaches have been successfully used to characterize targets of autoimmune Abs (7), allergy (8), or protective immunity (9). Such assays, however, have not addressed the effector potential of array-bound Abs so far. Functional tests would be particularly useful in the case of IgG, which in the human has four subclasses with different functions. Interestingly, isotype differences in the recognition of self-Ags within the same serum sample have been already shown (10). To circumvent the problem of detailed isotype, glycoform, and affinity determinations of Abs we have introduced the measurement of deposited complement component C3 fragments on Ag microarrays (11).

Components of the complement system circulate in inert forms that achieve activity by undergoing conformational changes induced by target recognition (C1q, MBL), proteolytic cleavage (C2, C3, C4, C5, fB), or by multiprotein assembly (C5b-C9 complex). C3, C4, and C5 belong to the α_1-macroglobulin family of proteins; C3 and C4 contain the characteristic internal thioester bond, while C5 has lost it. All three generate a small fragment, termed C3a/4a/5a, upon their first cleavage that exerts anaphylatoxic activity via receptors C3aR and C5aR. The highly reactive thioester bond that becomes exposed upon cleavage (12) binds to nearby nucleophilic groups, thereby covalently attaching C3b or C4b to macromolecules in the vicinity of activation. Surface-bound complement fragments serve as subunits of the C3 and C5 convertases and also as ligands of various complement receptors. Complement receptor (CR)[4] type 1 binds C3b and C4b with high affinity and helps immune complex removal by erythrocytes and phagocytic uptake by myeloid cells (13). CR2 on follicular dendritic cells captures immune complexes for storage and presentation, while B cell CR2 enhances Ag receptor-induced

*Immunology Research Group, Hungarian Academy of Sciences and †Department of Immunology, Eötvös Loránd University, Budapest, Hungary; ‡Department of Immunodiagnostics, National Medical Center, Budapest, Hungary; and §Diagnosticum Ltd., Budapest, Hungary

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[2] Current address: State Health Centre, Ministry of Defence, Podmaniczky utca 109-111, 1062 Budapest, Hungary.

[3] Address correspondence and reprint requests to Dr. József Prechl, Department of Immunology, Pázmány P.s. 1/C, 1117 Budapest, Hungary. E-mail address: jprechl@gmail.com

[4] Abbreviations used in this paper: CR, complement receptor; RFI, relative fluorescence intensity; SLE, systemic lupus erythematosus; SSA, Sjögren's syndrome Ag A; SSB, Sjögren's syndrome Ag B.

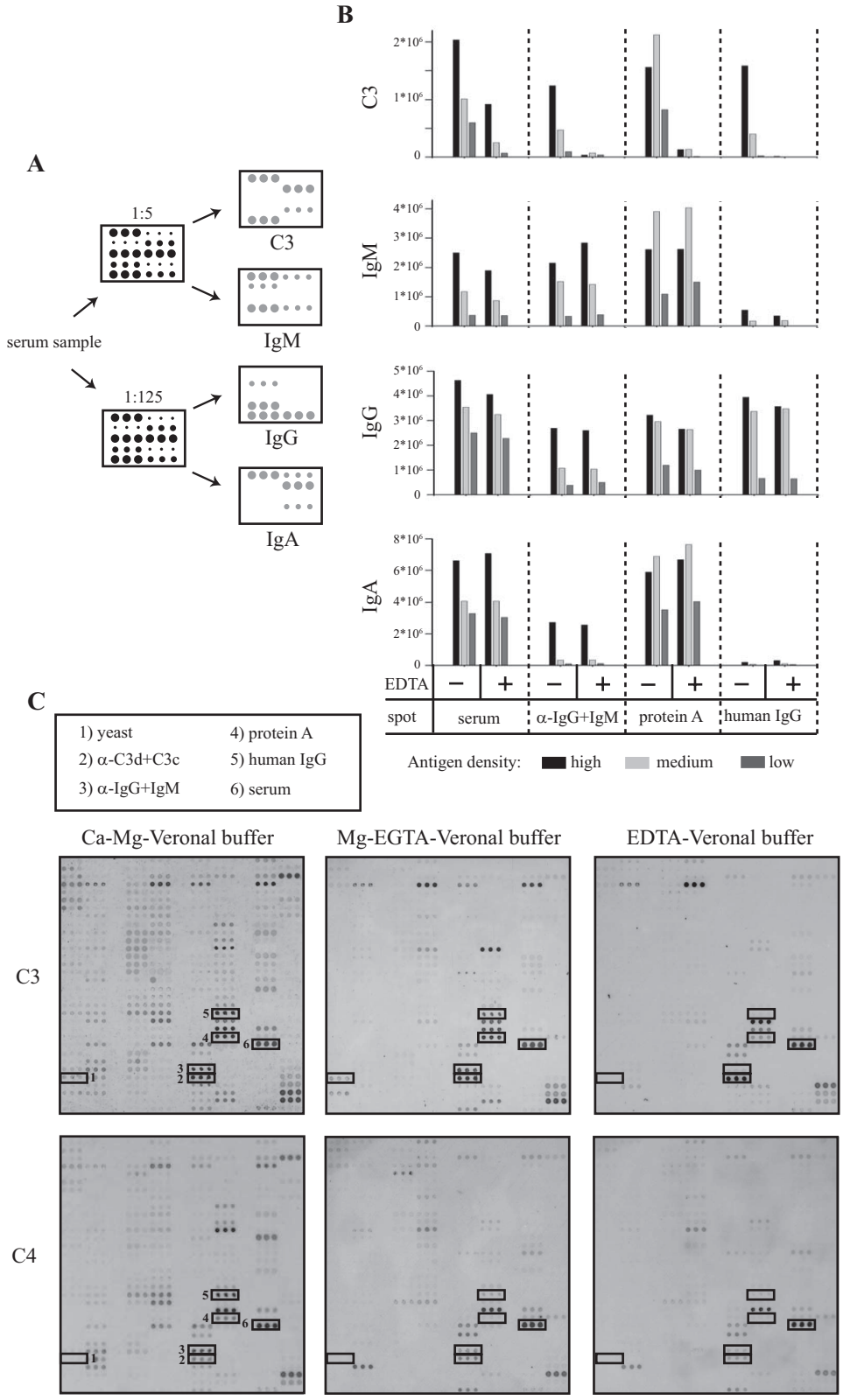

FIGURE 1. Experimental design and characterization of C deposition. *A*, Serum samples were diluted in Veronal buffer either 5-fold or 125-fold and applied to two identical Ag arrays. C3 deposition with IgM binding and IgG along with IgA binding, respectively, were detected on the arrays using fluorescently labeled secondary Abs. *B*, Deposition of C3 fragments reflects activation of C. C3, IgM, IgG, and IgA signals from the indicated four reference materials, each at three densities, are shown in the absence (−) or presence (+) of EDTA. Results are representative of three independent experiments. *C*, Serum samples were applied to Ag arrays in the presence of Ca^{2+} and Mg^{2+}, Mg^{2+} only, or in buffer without bivalent cations. In addition to C3 fragments, array-bound C4 was also determined. Results represent two independent experiments with identical results.

activation (14). CR3, CR4, and the recently described CRIg are primarily expressed on macrophages and dendritic cells and mediate phagocytosis (15–17). The extent and nature of complement activation thus has a modulatory effect on immune recognition and responses via numerous routes.

Although there is low rate of continuous breakdown of C3, this is greatly enhanced by immune complexes that can stimulate C activation and amplification of C3b generation by all three (classical, lectin, and alternative) pathways (18). The efficiency of C activation is influenced by several properties of the Abs forming the immune complex: affinity (19, 20), oligomerization (21), and glycosylation (22). In turn, the extent and nature of C activation has a modulatory effect on immune recognition and responses via numerous routes (23). We hypothesized that the combined

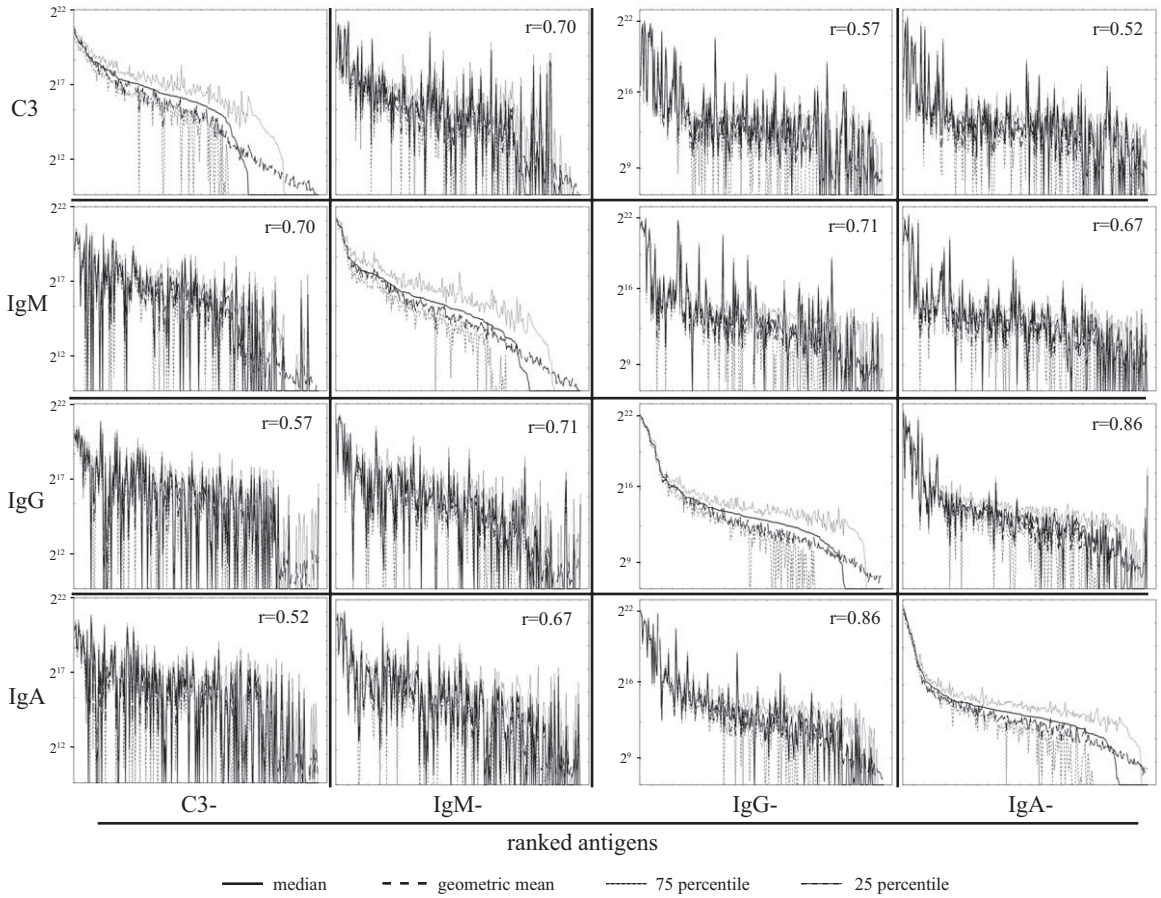

FIGURE 2. Distributions of ranked binding data. Medians, interquartile ranges, and geometric means of RFI signals from C3 fragment, IgM, IgG, and IgA measurements are shown. Data are derived from the measurement of 29 serum samples applied to arrays containing 85 materials, each at three dilutions. Spotted Ags were sorted according to the median of each measured parameter to visualize their distribution and relationships with each other. Correlation coefficients of medians, obtained excluding reference materials, are shown; all p values <0.001.

measurement of Ab binding and C deposition on Ag arrays may help obtain a functional view of the humoral response and make better predictions concerning its consequences. Indeed, using the two parameters of C3 deposition and Ig binding we were able to discriminate immune responses dominated by innate or adaptive components (24). Herein we present results describing the first application of on-chip complement activation in humans and a comprehensive comparison of Ag microarray-derived complement and Ab profiles. These findings suggest that measurement of human C3 deposition on Ag microarrays can provide information that is not extractable from IgM, IgG, or IgA binding data and helps the functional characterization of Ab responses.

Materials and Methods

Serum samples

Serum samples, provided by the National Medical Center from its repository or by healthy volunteers in our laboratory, were stored at −70°C until use. All serum samples were assigned a code number and, except for gender, no personal information was associated with array-derived data, in accordance with ethical regulations of the institute. Two sets of samples were examined in this study: 18 sera that were found to show signs of autoimmunity by classical screening tests (Abs against Sjögren's syndrome Ag B (SSB), Sjögren syndrome Ag A (SSA), dsDNA, Jo-1, anti-nuclear Abs, anti-mitochondrial Abs) and 11 sera that passed this screen. All samples were further characterized by an approved diagnostic array, CombiChip Autoimmune 1.0 (Whatman). Measurements were conducted according to the instructions of the manufacturer. Based on these results and on gender, two subsets of samples were selected to represent female controls ($n = 8$), with only negative or borderline values, and individuals with increased risk of systemic autoimmunity (AI, $n = 7$), with dsDNA reactivity and additional positive results. For the complete list of markers characterizing sera, see Table I.

Microarray production and measurements

Eighty-five different Ags (supplemental Tables III and IV)[5] were spotted onto homemade nitrocellulose-covered glass slides using a BioOdyssey Calligrapher miniarrayer (Bio-Rad). The data discussed in this publication have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (GEO) (25) and are accessible through GEO series accession no. GSE12943 (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc). Features were printed in triplicates of 1/5 serial dilutions then stored at 4°C in sealed bags. Dried arrays were rinsed in PBS for 15 min before use, then incubated with diluted serum at 37°C for 1 h, providing suitable conditions for C activation. For each subject 5- and 125-fold diluted serum was used in two different arrays for the detection of bound C3-IgM or IgG-IgA, respectively (see Fig. 1*A*). Sera were diluted in 5% BSA, 0.05% Tween 20, and Ca- and Mg-supplemented Veronal buffer. Alternatively, Veronal buffer with 2.5 mM $MgCl_2$ and 6.2 mM EGTA or Veronal buffer with 25 mM EDTA was used, as indicated in the text. Serum-treated slides were washed with 0.05% Tween 20 containing PBS, then incubated in the mixture of 1/2500 diluted FITC-conjugated rabbit anti-human C3d + C3c Ab (Dako) and 1/5000 diluted Cy5-conjugated $F(ab')_2$ fragment goat anti-human IgM (Jackson ImmunoResearch Laboratories) or 1/2000 diluted FITC-conjugated $F(ab')_2$ fragment goat anti-human IgG (Axell) and 1/5000 diluted Cy5-conjugated $F(ab')_2$ fragment goat anti-human IgA (Jackson ImmunoResearch Laboratories). In some experiments FITC-conjugated goat anti-human C4 from MP Biomedicals was used at 1/2000 dilution. Labeling with fluorescent Abs was conducted

[5] The online version of this article contains supplemental material.

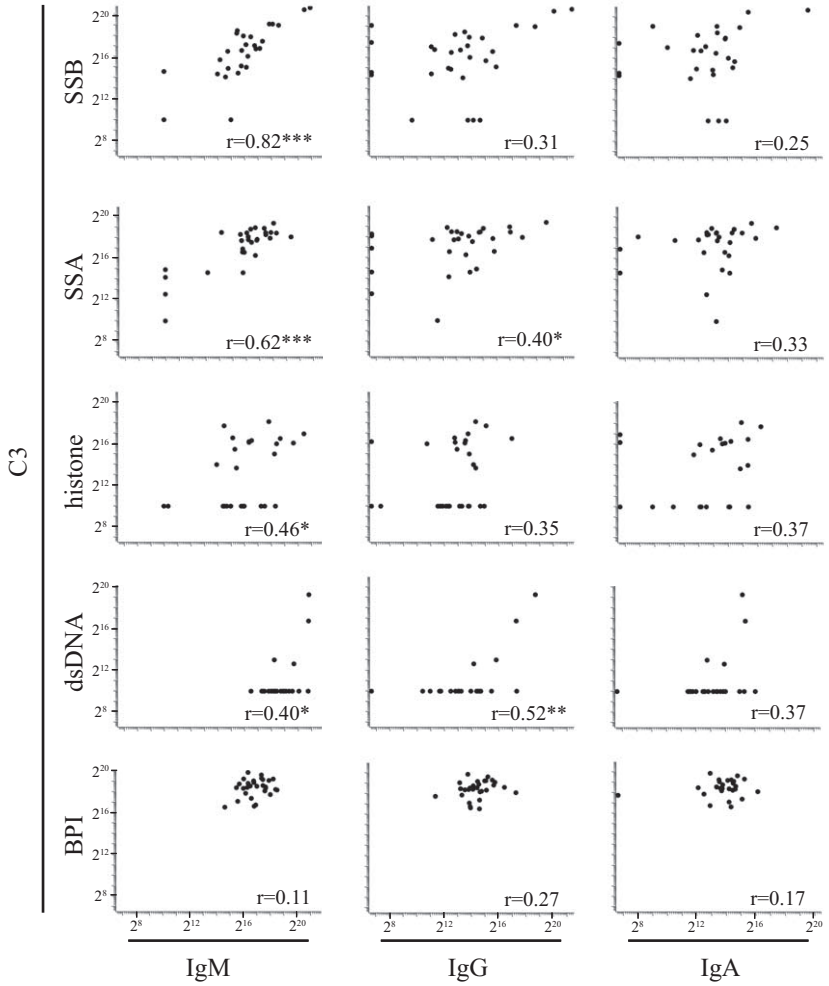

FIGURE 3. Typical correlations of Ab binding and C activation by representative autoantigens. Plots depict correlations of signals of the highest Ag density, with each spot representing a particular serum sample. The complete list of correlations is available in supplemental Table V. BPI indicates bactericidal/permeability increasing protein. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

at room temperature for 30 min in 5% BSA and 0.05% Tween 20 containing PBS. Following washing in 0.05% Tween 20 containing PBS, arrays were dried and scanned on Typhoon Trio+ imager (Amersham Biosciences).

Analysis of microarray data

Data were analyzed with ImageQuantTL (Amersham Biosciences) software. Signal intensities were calculated by subtracting background from medians of three parallel signal intensities in a spreadsheet program (Microsoft Excel). For interassay comparison, data were normalized to the mean of two dilutions of selected control materials. Protein G, anti-human IgG + IgM, human IgG, and protein A features on arrays were used for normalization of C3, IgM, IgG, and IgA signals, respectively. Only data derived from slides with a normalization factor between 0.4 and 2.5 were considered suitable for analysis. Based on the distribution of data, normalized fluorescence intensity signals <1000 for C3 and IgM or <100 for IgG and IgA were rounded up to these values.

Statistical analysis

Spearman rank correlations were calculated for comparing C3, IgM, IgG, and IgA signals using Statistica AGA software. *k*-means clustering with 10 iterations for obtaining two clusters was conducted for the characterization immune profiles. A Mann-Whitney *U* test was used for assessing significance of differences between normal and systemic lupus erythematosus (SLE) risk serum samples, and an *F* test was used for determining differences between clusters. $p < 0.05$ was considered statistically significant.

Results

Incubation of Ag microarrays with serum results in activation-dependent deposition of complement C3 fragments

We tested deposition of C3 on an array containing 85 different materials including self- and foreign Ags and reference materials (for complete list and positions, see supplemental Tables III and IV). In addition to C3 fragments, we also measured the binding of serum Igs with C activating ability, namely IgM, IgG, and IgA (Fig. 1*A*). To confirm that C activation is required for the binding of C3 fragments to the Ags, we chelated Ca^{2+} and Mg^{2+} ions with EDTA and compared signals on four reference spots. Human serum, spotted as a positive control, contains intrinsic C3 that is detectable even when EDTA containing serum sample is applied to the chip, although signals are reduced, indicating that C3 fragments are deposited when normal serum is applied to the chip. Spotted human IgG activates complement because of the conformational alterations induced by adsorption to the microarray surface. Polyclonal anti-human Ig Abs and the bacterial superantigen protein A activate C indirectly via the capture of Igs from the applied serum sample. C3 deposition was inhibited by EDTA, while binding of serum Igs was not influenced (Fig. 1, *B* and *C*).

To further characterize the events leading to deposition of C3 fragments, we measured microarray-bound C4 and the effect of selective inhibition of the classical and lectin pathways by Ca^{2+} chelation. As shown in Fig. 1*C*, C3 and C4 signals from most of the Ags disappeared when only the alternative pathway was allowed to proceed. Exemptions are the known potent alternative pathway initiator, yeast, and some control materials. Interestingly, spotted (human IgG) or captured (protein A, anti-IgG + IgM) Igs also effectively induced C3 deposition by the alternative pathway. Most Ag-derived C3 signals are therefore derived from classical or lectin pathway-initiated C activation.

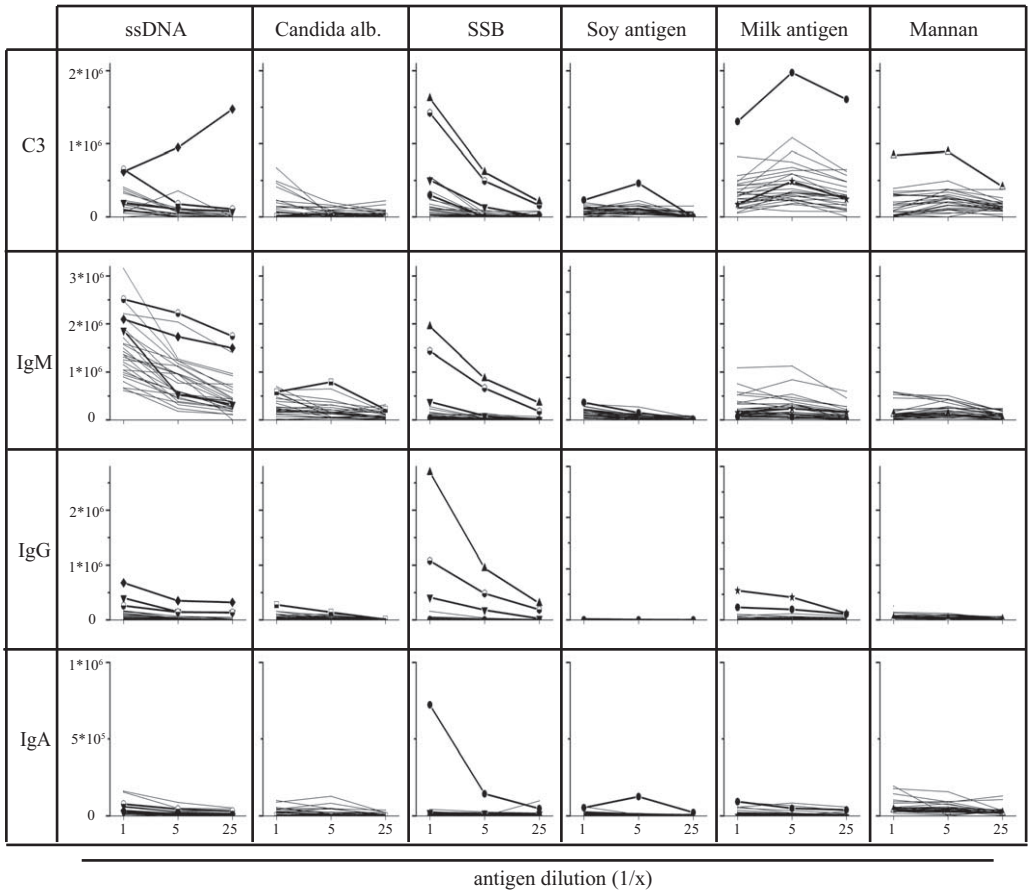

FIGURE 4. C-activating potential of Abs is highly variable individually. RFI signals of all three densities of six representative Ags are shown. Serum samples showing extreme behavior in one or more parameters are highlighted for each Ag.

Overall complement activation is correlated with Ag recognition by Abs

Abs of the IgM, IgG, and IgA class represent >99% of plasma Igs. IgD and IgE concentrations are lower by two or three orders of magnitude; additionally, these Igs are incapable of C activation. Thus, by measuring the binding of IgM, IgG, and IgA we examined all Abs that could potentially contribute to C3 fragment deposition on the arrays.

For the overall characterization of C deposition we ranked Ags according to the medians of relative fluorescent intensities (RFI) of each of the measured molecules (Fig. 2). Distribution of C3 data was comparable to that of IgM, IgG, and IgA measurements, indicating that the Ags used generally show variable abilities of C activation on a scale similar to Ab binding. Interquartile ranges enclose datasets, which likely reflect binding of natural Abs and C deposition. Displaying Ab binding on the C3 ranked data (Fig. 2, *first column*) implies, however, that C activation is not necessarily accompanied by binding of a certain Ab class. These fluctuations are a sign of conflicting C3 deposition and Ab binding data and are most pronounced when comparing C3 and IgA data and are least observed for C3 and IgM comparison. C3 deposition was correlated with IgM, IgG, and IgA binding in this order, and IgG binding was strongly related to IgA and to a lesser extent to IgM.

Ag- and individual-dependent variations exclude reliable prediction of the extent of complement activation by measurement of Ab binding

When measuring multiple Ags in multiple samples, there are two possibilities for expressing C3 deposition as a function of Ab binding: depicting the position of the same Ag in all samples or plotting all Ags for a given individual. Fig. 3 illustrates the former representation for five autoantigens that are each representative for a potential dominant isotype influence. As implied by Fig. 2, IgM binding had major influence on C3 fragment deposition in most of the cases (Fig. 3, SSB). Of the tested Ags, 65% showed significant positive correlation between C3 deposition and IgM binding, with r in the range of 0.37–0.82 (for a complete list of correlation coefficients, see supplemental Table V). Importantly, however, the presence of relatively high amounts of IgM was not necessarily accompanied by C activation (compare dsDNA with BPI (bactericidal/permeability increasing protein) in Fig. 3). This suggests that considerable differences can exist in C-activating ability of Ab species of the same class. For some Ags, such as SSA and dsDNA, both IgM and IgG binding were correlated with C3 deposition; C3 signals were positively correlated with IgG in 9% of the Ags and with IgA in <5% of them. This form of data representation also allows the identification of serum samples with unusual properties, like those showing C activation by dsDNA in Fig. 3.

Despite the characteristic correlations between C and Ig for each Ag, individual variations preclude reliable prediction of C activation simply by measuring Ig binding. This is further exemplified by Fig. 4, which shows data obtained from all three measured Ag densities. While increased reactivity of any of the three Ig classes can result in increased C activation, such increase could also be observed without substantial increase in C deposition (see milk, candida, and ssDNA in Fig. 4). Conversely, extreme C3 deposition was also observed without any (see mannan in Fig. 4) or with a modest (see milk and ssDNA in Fig. 4) rise in Ig binding.

Table I. *Classification of samples based on gender and autoantibody levels*

Patient No.	Gender	SSA/Ro-52	PR3	SSA/Ro-60	MPO	SSB/La	dsDNA	Mi-2	PM/Scl-75	CENP-B	PM/SCL-100	U1–70K	Jo-1	Sm	Scl-70	Group[a]
1000	♀	−[b]	−	−	−	−	−	−	−	−	−	−	−	−	−	
2000	♂	−	−	−	−	−	+	+	−	−	−	±	−	−	−	
2988	♀	−	−	−	−	−	+	−	−	+	−	+	−	±	+	AI
2989	♀	−	−	−	−	−	+	−	−	+	−	+	+	−	−	AI
3024	♀	−	−	−	−	−	−	−	−	−	−	−	−	−	−	C
3773	♀	+	−	+	−	+	+	+	−	+	+	+	−	−	−	AI
3885	♀	+	−	+	−	+	−	+	±	+	+	±	−	±	±	
3899	♀	+	−	−	−	+	−	−	−	−	−	−	+	−	−	
3936	♀	−	−	−	−	−	−	−	−	−	−	+	−	−	−	
3937	♀	−	−	−	−	−	−	−	−	−	−	−	−	−	−	C
3951	♀	+	−	+	−	+	±	−	−	+	±	−	−	−	−	
4199	♀	−	−	−	+	−	±	−	−	+	−	−	−	−	−	
4211	♀	−	−	−	−	−	+	−	−	±	−	+	−	+	−	AI
4249	♂	−	−	−	−	−	−	−	−	±	−	−	−	−	−	
4248	♀	−	−	−	−	−	−	−	−	−	−	±	−	−	−	C
4624	♀	+	−	+	−	+	+	−	−	±	+	−	−	−	−	AI
5000	♀	−	−	−	−	−	−	−	−	−	−	−	−	−	+	
5066	♀	±	−	−	±	−	±	−	−	+	±	+	−	−	−	
5134	♀	−	−	−	−	−	−	−	−	−	−	−	−	−	−	C
5143	♀	−	−	−	−	−	−	−	−	−	−	+	−	−	−	
5381	♂	±	−	−	−	+	+	−	−	−	−	−	−	−	−	
5415	♀	−	−	−	−	−	−	−	−	−	−	−	−	−	−	C
5982	♀	−	−	−	−	−	+	−	−	+	−	−	−	−	−	AI
6000	♀	−	−	−	−	±	−	−	−	±	−	−	−	−	−	C
6196	♀	±	−	−	−	+	+	−	−	+	−	±	−	−	−	AI
6209	♀	−	−	−	−	−	−	−	−	±	−	+	−	−	−	
6223	♀	−	−	−	−	±	−	−	−	−	−	−	−	−	−	C
6248	♀	−	−	−	−	+	−	−	−	±	−	+	−	−	−	
6277	♂	−	−	−	−	−	+	−	−	−	−	−	−	−	−	
6311	♀	−	−	−	−	−	−	−	−	−	−	−	−	−	−	C

[a] C, control; AI, systemic autoimmunity risk.
[b] Test results were −, normal range; ±, borderline; +, elevated.

Functional Ab profiles can help characterize immune status of individuals and disease

Based on their Ig-binding and C-activating properties in normal human serum, Ags can be positioned in two-dimensional spaces derived from Ig-binding and C deposition data. Changes in the coordinates would imply altered reactivity and/or functionality of Ag-specific Igs in the analyzed serum sample. To test whether such changes could be indicative of disease status, we selected two subsets of samples based on laboratory results predicting the presence of autoimmune disorder (Table I). Samples with negative results were considered controls, and samples with reactivity against dsDNA and at least one more nuclear Ag were considered being at high risk for systemic autoimmunity. Grouping-related changes in the coordinates of six Ags, used in the serodiagnostic process of systemic autoimmune disorders, are shown in Fig. 5, *A–C*. To confirm that changes reflect true segregation of data, we performed *k*-means clustering and obtained coordinates of centroids representing two clusters (Fig. 5, *D–F*). The pattern of changes observed by categorization and by clustering is similar for histone, ssDNA, dsDNA, and nucleosome Ags when C3 and IgM or IgG signals are examined. SSA and SSB cluster differently from the generated groups, suggesting that these Ags are not reflecting our grouping criteria. Importantly, the C3/IgG ratio significantly increased for nucleosome with a concurrent decrease of C3/IgM for histone, indicating complex changes in the immune reactivity against nuclear components. With the exception of histone, which showed decreased IgM reactivity in the SLE risk group, patterns for the three Ig classes were similar.

To examine the potential clinical relevance of complement measurements, diagnosis of the selected autoimmune subjects were obtained, as shown in Table II. Complement activation by dsDNA-specific Abs was only observed in the three subjects with SLE but not others who had lower but still elevated anti-dsDNA Ab reactivities without complement deposition.

Discussion

In the body, Ag recognition by Igs and the formation of immune complexes are immediately followed by interactions with soluble or cell-bound components of the immune system. One of these interactions, C activation, can be monitored by the measurement of C3 fragments that covalently attach to the site of C activation. Immersing an array of Ags into serum therefore allows the specific identification of Ags that capture Abs and/or activate C (Fig. 1). Our results indicate that, with a few exceptions, the alternative pathway alone was not responsible for the observed C3 deposition patterns (Fig. 1*C*). On the other hand, despite the general correlation between C3 and C4 signals, the latter were much weaker. This was also the case with C1q signals (data not shown). Taken together, these data suggest that alternative pathway plays a minor role in the initiation of C3 deposition, and although the Ig-initiated classical (or lectin) pathways are responsible for the initiation of complement activation, C1q and C4 binding was close to detection limits. Due to the presence of the amplification loop, C3 can actually be more abundant at complement activation sites. These data therefore do not rule out that classical or lectin pathway-initiated generation of C3b serves for alternative pathway convertase generation and that the alternative pathway amplifies classical or lectin pathway-triggered C3 deposition.

Ags that captured more Abs were generally more prone to C3 deposition, and the binding of IgM was especially well correlated with the presence of C3 fragments (Fig. 2). Looking at the Ags separately confirmed that IgM binding had the strongest effect on

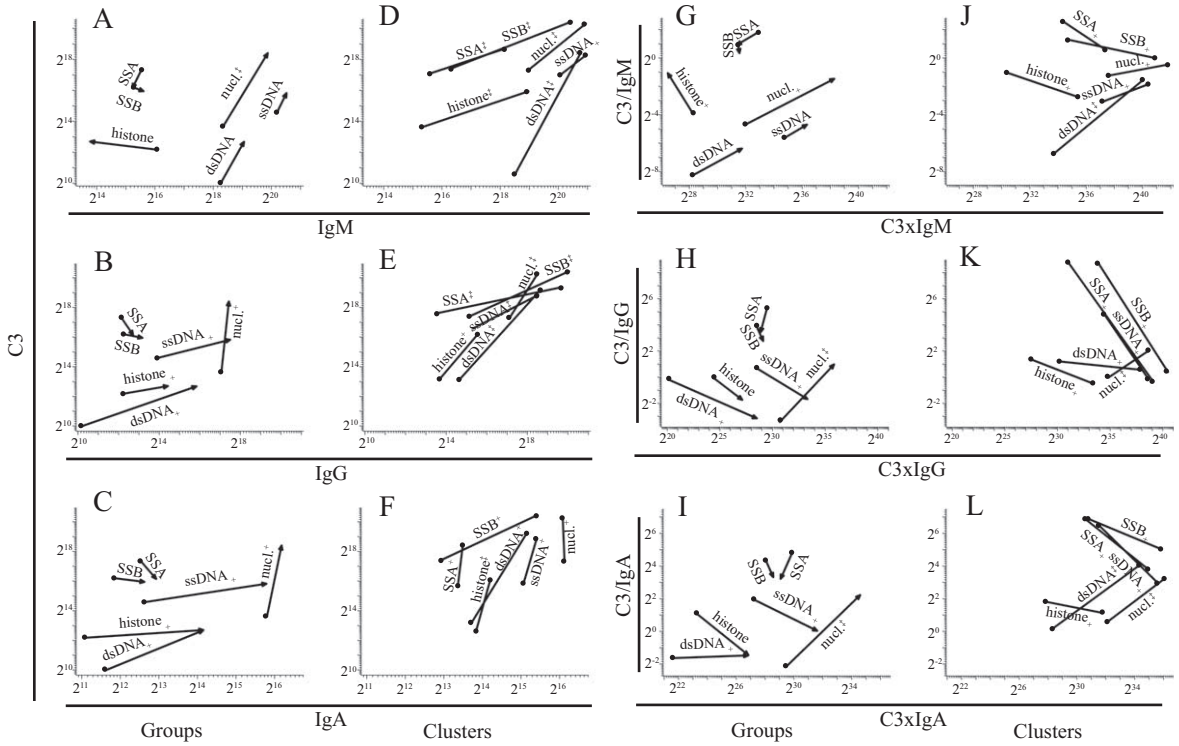

FIGURE 5. Visualization of disease-associated alterations in two-dimensional immune profiles. Ag array data for six Ags, which are used in SLE diagnosis, are depicted in two-dimensional representations, incorporating derivatives of C3 deposition and Ig binding values. Two subsets of patients (control, $n = 8$; autoimmune, $n = 7$) were selected according to criteria described in *Materials and Methods*. *A–C* and *G–I* show connected geometric means of signals of normal (●) and autoimmune risk samples (arrowhead). Using *k*-means clustering, two clusters for each dataset were identified. Centroids of these clusters are shown by connected circles in diagrams *D–F* and *J–L*. Changes in dsDNA coordinates reflect and confirm categorization criteria. Significant change ($p < 0.05$) along the *y*- or *x*-axis is indicated by upper (Ag^{+}) or lower (Ag_{+}) index plus sign, respectively; $Ag^{\ddagger}$ stands for significant alteration along both axes. Nucl. indicates nucleosome.

C, followed by IgG. This observation is in line with the potent C-activating potential of IgM (21), although IgM has also been shown to inhibit C activation (26, 27) or ameliorate disease (28, 29). Actually, all Ags showing significant correlation between IgG and C3 fragment values also showed significant IgM correlation. However, signatures characterizing individuals, that is, statistical outlier values, were often not predictable by counting on these correlations (Fig. 5). These data suggest that for most of the studied Ags threshold levels of C deposition are set by the amount of bound IgM molecules, which can be components of the natural Ab repertoire. High-affinity binders of any class, induced as part of an immune response, can then either further increase these levels or leave them unchanged, depending on the nature of the Ab. Therefore, we propose that the measurement of C-activating properties of sera, reflecting mainly the characteristics of serum Abs, can help to functionally characterize Abs using Ag arrays. An ideal pair for the profiling would be C3 and IgG since most protective or pathogenic Abs belong to this class. Additionally, when using Ag panels known to induce mucosal type responses, IgA can also be utilized since this Ig class can also initiate C activation (30).

Table II. *Relationship between complement activation and clinical diagnosis*

Patient No.	Clinical Diagnosis	dsDNA IgG (RFU)	dsDNA C3 (RFU)
2988	Primary biliary cirrhosis	25,167	1,000
2989	Primary biliary cirrhosis	26,033	1,000
3773	SLE	419,885	617,163
4211	Idiopathic anemia	21,089	1,000
4624	SLE	57,090	7,872
5982	SLE	158,304	105,869
6196	Scleroderma	44,984	1,000

To confirm that Ag-specific measurement of C activation can help in identifying disease-associated Ab signatures, we compared functional, two-dimensional profiles of two subsets of subjects categorized by the absence or presence of anti-nuclear autoantibodies associated with systemic autoimmunity. C deposition was significantly increased in the autoimmune group on nucleosomes, an early diagnostic marker of SLE. Anti-nucleosome Abs were found to be correlated with disease activity and C3 consumption (31); this latter observation can be accounted for by the potent C activation of these Abs as observed in our study (Fig. 5, *A–C*). Abs against SSA and SSB are found in patients suffering from various connective tissue diseases, which is the probable reason for discrepancy between the anti-dsDNA Ab-based grouping and clustering results for these Ags. Although there was a tendency of increased C deposition for histone, ssDNA, and dsDNA (Fig. 5), this was exceeded by the increase in the reactivity of IgG, resulting in no significant change of C3/IgG ratios (Fig. 5*H*). Of note, among the subjects showing increased dsDNA Ab reactivity, C3 fragment deposition was only detected in SLE patients. Notwithstanding the underlying mechanism of disease, virtually all autoimmune diseases are associated with circulating autoantibodies, which bind self-structures. For many diseases these autoantibodies are found in serum samples many years before disease onset. By dissecting the functionality of these Abs we may obtain more precise markers of disease prognosis.

In this study we have chosen to examine C3 deposition since it represents the point of convergence of the three C pathways and therefore allows their integration with Ab binding measurements. Furthermore, cleavage fragments of C3 are all potent activators of the C receptors C3aR, CR1, CR2, CR3, and CR4. Alternatively, the deposition of C4, a process very closely resembling that of C3 (32), could also be measured in an identical fashion. Deposition of C4 reflects activity of the classical and lectin pathways, thereby providing information that is more specific for the nature of the C initiation. Comparison of C3 and C4 deposition profiles is also expected to supply interesting information about Ag-host interactions.

For the mapping of immune response profiles a two-dimensional immunological map was proposed recently (33) with inflammatory-regulatory and Th1-Th2 axes. The pleiotropic cytokine IL-6, which is associated with the inflammatory axis, is an inducer of C synthesis (34); C3 production increases many fold during acute phase reaction. Additionally, Ab isotypes associated with the inflammatory axes IgG1 and IgG3 in humans are potent activators of C. Consequently, functional Ab signatures with relatively strong C deposition are likely to imply an Ag-host relationship that is skewed toward inflammation. The role of C in inflammatory disorders is well-appreciated and far more complex than a simple proinflammatory action (35). Monitoring C activation in Ab profiling assays can therefore promote our understanding of the multifaceted role it plays in disease pathogenesis.

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Disclosures

Eötvös Loránd University and the Hungarian Academy of Sciences have a patent pending on Ag microarray-based complement activation measurements.

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