From Pauling’s abzyme concept to the new era of hydrolytic anti-DNA autoantibodies: a link to rational vaccine design? – A review

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Abstract: Specific entities of naturally-occurring DNA hydrolytic/cytotoxic antibodies (abzymes) are linked to autoimmune and lymphoproliferative disorders. Suggested sequence of underlying activities conform to such entities penetrating the living cells, trans-locating to nucleus and recognising specific binding sites within single- or double-stranded DNA. Their origin is unknown since corresponding immunogens are unidentified. These anti-DNA antibodies could be the organism’s immune response to microbial attack. Their structure, function and pathogenicity were investigated in wet-lab and via bioinformatics in context of Rational Vaccine Designs. This paper offers a comprehensive critical review on the subject in the light of known and newly proposed concepts.

Keywords: autoimmune diseases; human immunodeficiency virus; anti-DNA antibodies; DNA vaccine; RVD; rational vaccine design.

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1 **Introduction**

Natural and artificially-developed, highly-efficient catalytic antibodies (abzymes) with manufacturing feasibility indicate potential applications like evolving antibodies required in human therapy and preventing (via vaccination) cancer, AIDS, autoimmune diseases and so on (Taguchi et al., 2008). Within the ‘cryptic soul’ of autoimmune dysbalance, evolution of such antibodies is akin to evolving enzymes except that, enzyme evolution bears a long history, while the binding affinity of antibody development spans just weeks. Relevant research suggests that a catalytic antibody-based approach could be a key tool in selective chemotherapeutic strategies against some cancers and HIV-1 (Planque et al., 2008).

Naturally-occurring DNA hydrolytic anti-DNA antibodies are apparently linked to various autoimmune and lymphoproliferative disorders, like systemic lupus erythematosus (SLE), multiple sclerosis, Sjogren syndrome, B-chronic lymphocytic leucosis and multiple myeloma. The origin of antibodies is unknown, and B-cell population linked to the production of antibodies with catalytic and cytotoxic functions against double-stranded (ds) or single-stranded (ss) DNA is yet to be identified. Suggested sequence of underlying activities conform to penetration of antibodies (recirculation after secretion) into living cells, translocation to the nucleus and recognition of specific binding sites within the DNA (ds or ss) (Shuster et al., 1992; Alarcon-Segovia, 2001).
Whether hydrolysis is a sequence-specific event is questionable. The interplay between anti-DNA antibodies and different types of DNA has not yet been elucidated, though anti-DNA antibodies with DNA hydrolytic capacity could be an organism’s immune response to microbial attack. Targeted for neutralisation are microbial DNA and/or specific genes within the microbial DNA sequence (Pisetsky, 2009; Pavlovic, 2009).

Computational methods enable knowing abzyme specificities adjunct to trial-and-error wet-lab experiments saving time and money. Relevant tools are appropriate in the autoimmunity analyses of binding-sites (the ‘hot spots’ of antibody attraction within microbial DNA), binding activity and predictions toward most probable motifs for antibody-DNA binding as well as modelling hydrolytic kinetics (Ponomarenko et al., 1999).

Notwithstanding the traditional DNA vaccines (based on protein antigens), making of Rational Vaccine Designs (RVDs) could benefit from computational predictions of protein (Bublil et al., 2006) or even DNA epitopes, (for example, as needed in for an Ebola virus vaccine). Relevant epitope is a component of the binding protein of relevant viral ssDNA known through wet-lab experimentation (Banton et al., 2010).

Any motif, which the immune system is trained to recognise, could be a vaccine target. Knowledge on molecular structures and functions of immune systems allows hypothesising that DNA vaccines could act through mechanisms differently than, known classical pathways. Further, anti-DNA antibodies could act principally in RVDs of certain autoimmune diseases. In conclusion, computationally-decided RVD might be useful in optimised, crucial or adjuvant problem-solving and time-saving approach for efficient DNA vaccination (in cases where protein-based microbial vaccines have failed as in HIV and human Parvovirus B19-induced diseases) (Taguchi et al., 2008; Pavlovic et al., 2010).

2 Catalytic antibodies: definition and evolution

Linus Pauling in 1940 suggested that enzymes can cause appreciable rate accelerations by preferentially binding the transition-state complex formed by the reaction (Pauling, 1940, 1946). Enzymes perfect their abilities over the evolutionary scale and assume efficient structures for catalysis (Takahashi et al., 2001). But antibody-binding sites could evolve toward efficient structures (for binding) just in weeks during the process known as affinity maturation (Pollack et al., 1986).

Antibodies are elicited when a host is infected with a bacterium or virus (Jerne, 1942). The offended organism produces antibodies with binding sites exactly complementary to some molecular feature of the invader (microbe). Ordinarily, antibody molecules simply bind without catalysing reactions. They recognise and bind only to the invader, identifying it as foreign and prompt its destruction by the immune system (Pollack et al., 1986).

Catalytic antibodies are produced when animals are immunised with hapten molecules specially designed to elicit antibodies containing binding pockets capable of catalysing chemical reactions with increased reaction selectivity due to lowered energy for the desired reaction, and other geometrical aspects/charge distributions that lead to unwanted products are prevented.
Another antibody-specific concept (of Figure 1) refers to idiotypic network theory (Jerne, 1942, 1984; Shoenfeld, 2004). Relevant concept states that an antibody raised against the combining site of a first antibody may contain information regarding the three-dimensional structure of the original antigen. Thus, if an active site of an enzyme is used as the antigen, the properties of internal imagery of anti-idiotypic antibodies can be exploited to design catalytic antibodies (Blank and Shoenfeld, 2008). Early experiments generated esterolytic antibodies using phosphonate/phosphate transition state analogues (Kafarski and Lejczak, 1991). Recent studies focus on chemical screens and genetic-/phage-based selections in identifying mutants with enhanced catalytic functions to engineer antibodies with anti-cancer and anti-viral activities. A catalytic antibody called 38 C2 is now applied to a wide range of anti-cancer drugs.

Certain antibodies engineered recently can also recognise diverse viral forms found across the world solving the problem of viral changeability. Unlike regular antibodies, these abzymes degrade the virus permanently. Clinical RV144 trials show that a combination of two antibodies components can cut the risk of HIV infection by 31% (Grant and Bass, 2010).

3 Anti-DNA hydrolytic autoantibodies: the role in normalcy and pathology

Anti-DNA antibodies are naturally-occurring, non-manufactured DNA-binding proteins that belong to immunoglobulin M (IgM) or G (IgG) classes. Models describing the origin of DNA-hydrolysing catalytic antibodies, ‘DNA-abzymes’, though proposed (Ceppelini et al., 1957), no explanations on unique aspects of anti-DNA antibody origin exist. Some of these antibodies may have emerged inadvertently during the course of a normal immune response due to their induction by antigens-bearing structures mimicking the DNA (mimotopes). The mimotope is closely related to epitopes responsible for idiotypic antibody and appears to be cryptic or normally hidden in the native protein or DNA. It can be further surmised that even naturally-occurring anti-DNA non-hydrolytic antibodies could serve as a template for the production of anti-DNA catalytic antibodies within an idiotypic network. Possible alternatives
include epitope spreading, bystander and polyclonal activations (Kivity et al., 2009; Agmon-Levin et al., 2009; Israeli et al., 2009), though none of them are proven.

Naturally-occurring anti-DNA antibodies in healthy individuals do not generally exhibit sequence specificities in binding to DNA, and they do not have hydrolytic activity though, binding is a pre-requisite for hydrolysis (Nevinsky et al., 2000; Pavlovic et al., 2010). In Shuster et al. (1992) reported that some autoantibodies derived from patients with SLE possess DNA nicking activities (cytotoxic features) as confirmed in Nevzorova et al. (2003). The presence of anti-dsDNA antibodies in sera is diagnostic of SLE. Both anti-dsDNA and anti-ssDNA antibodies have been shown to deposit in patient tissues and glomeruli involving DNA binding (Spatz et al., 1997).

Hydrolytic anti-DNA antibodies classified as binding-specific for ssDNA and dsDNA (Spatz et al., 1997; Tanner et al., 2001) have a preference for certain DNA sequences like poly (dT) or poly (dG-dC) sequences (Spatz et al., 1997). Binding kinetics and specificities of anti-DNA antibodies are sparsely addressed (Pavlovic et al., 2009), and studies indicate that isolated human lupus polyclonal anti-DNA antibodies are capable of hydrolysing a modified version of Gololobov’s ssDNA sequence with a similar motif to human Parvovirus B19 (Pavlovic et al., 2010). Hence, elucidating the role of anti-DNA antibodies in certain autoimmune diseases is important in realising RVDs.

4 DNA as immunogen

Estimation of in vitro plasma DNA concentration shows marked increase in various pathological states (Steinman, 1979; Swarup and Rajeswari, 2007). Further, in a study (Pavlovic et al., 2007) using ELISA plates covered with oligo-dT, it is found that in sera of SLE patients positive for anti-ssDNA antibodies, the absolute concentration of these antibodies is higher in SLE (489.32 µg/ml), somewhat lower in B-CLL (301.18 µg/ml). In healthy individuals having positive antibodies, the concentration is the lowest (66.55 µg/ml). Varying DNA concentration is seen in certain types of cancer and autoimmune diseases (Giacona et al., 1998). Relevantly, other questions are

- Is non-immunogenic self-DNA with base monotony a genetically-active part of human DNA?
- Does an age-related increase in frequency of unmethylated motifs make human (mammalian) DNA more immunogenic?
- If so, what could be the cause of childhood SLE and other childhood autoimmune diseases?
- Or, does the immunogen microbial (viral or bacterial) DNA with more base pair and CpG islands variations make it more immunogenic?

Knowing whether autoimmune diseases are consequences of infection and, if so, whether hydrolytic anti-DNA autoantibodies would depict an organism’s response to microbial attack is to be ascertained. Further, antibodies being a natural component of immune response, whether antibodies of both ds- and ssDNA are pathogenic and whether hydrolysis is the associated mechanism (or at least a factor) influencing the pathogenicity has to be decided.
Microbial DNA (bacterial and viral) is a known immunogen, and it is suggested that a high occurrence of unmethylated CpG motifs in microbial DNA acts as a stimulator of anti-DNA antibody production and relevant flare of symptoms seen in lupus. The CpG motifs act through binding to transmembrane and cytosolic toll-like receptor 9 (TLR-9) on B-cells and plasmacytoid dendritic cells (Christensen et al., 2005; Kumagai et al., 2008). Some studies show that anti-DNA antibody binds to peptide mimicking synthetically-designed antigens, mimic viral or bacterial proteins. The spectrum of lupus symptoms and the heterogeneous nature of the disease suggest that it is probably caused and triggered by multiple environmental factors (similar to carcinogens) and therefore, requires individual diagnosis, therapy and prevention (Harley and James, 2006; Kalsi et al., 1999; Pavlovic, 2009).

Existence of anti-DNA antibodies to both ss- and dsDNA has queries on entities and their modality of presentation to immune systems. Beside the mechanisms already mentioned, the possibility of DNA (or a part of it) being directly involved is to be considered. The size of a DNA is a problem for antigen presentation. Hence, it is believed that what is presented to the immune system in the case of responsive anti-DNA antibody secretion is not DNA itself, but some cross-reactive, peptide mimicking antigen bound to DNA, RNA bound by protein, a smaller DNA-mimicking protein or its epitopal part. Controversies regarding this issue have not yet been resolved; but, relevant protein regarded as an epitopal candidate within Ebola virus DNA in bioinformatic perspective is indicated (Banton et al., 2010).

Recent evidence (Muruve et al., 2008) suggests that DNA or its features could be presented to the immune/innate immune system molecular components not previously known or implicated. But how DNA is presented to the immune system could be interesting. Recognition of pathogens by innate immune system is mediated by a set of germline-encoded receptors referred to as Pattern-Recognition Receptors (PRRs). They recognise conserved molecular patterns (pathogen-associated molecular patterns) shared by large groups of microorganisms. TLRs function as the PRRs in mammals plays essential role in the recognition of microbial components (Christensen et al., 2005). Signalling through TLR is a multi-step process. It involves transmembrane and intracellular TLRs and DNA-sensing proteins (Muruve et al., 2008) related to B-memory cell production. TLR-9 may interact with DNA and unmethylated CpG motifs (present in bacterial DNA) and might translate/transduce a DNA image of larger pieces of DNA.

Specific to RVD is the discovery of synthetic oligodeoxynucleotides containing unmethylated CpG motifs. They mimic immunostimulatory activity of bacterial DNA and induce strong T helper (Th) 1-polarising immune responses promoting cellular immunity (Table 1). Such nucleotides are promising vaccine adjuvants in immunotherapies for treatment of human diseases like cancer and so on.

Table 1  The effect of CpG DNA on various immune cells

<table>
<thead>
<tr>
<th>B-cells</th>
<th>Macrophages</th>
<th>Dendritic Cells (DC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6, IL-10 and IG</td>
<td>TNF α, IL-12, IL-6, NO</td>
<td>TNF α, IL-12, IL-6</td>
</tr>
</tbody>
</table>
Table 1  The effect of CpG DNA on various immune cells (continued)

<table>
<thead>
<tr>
<th>Expression</th>
<th>B-cells</th>
<th>Macrophages</th>
<th>Dendritic Cells (DC)</th>
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<tbody>
<tr>
<td>MHC Class II</td>
<td>MHC Class II</td>
<td>MHC Class II</td>
<td></td>
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<tr>
<td>CD80</td>
<td>CD80</td>
<td>CD80</td>
<td></td>
</tr>
<tr>
<td>CD86</td>
<td>CD86</td>
<td>CD86</td>
<td></td>
</tr>
<tr>
<td>Proliferation</td>
<td></td>
<td></td>
<td>Enhance antigen presenting activity</td>
</tr>
<tr>
<td>Become apoptosis resistant</td>
<td></td>
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5  Hydrolytic and cytotoxic anti-DNA antibodies

Structure, recognition and binding: Antibodies to DNA serving as models to study protein–DNA recognition (Ponomarenko et al., 1999) can help developing model systems to study protein–DNA recognition and computational approaches to RVD. Associated DNA crystal structures indicate that DNA binding causes significant conformational changes in the antibody casting a doubt whether an antibody–DNA interactions can be inferred from models of unliganded antibodies especially those that bind ssDNA. This is because PDB contains only a small extent of protein–ssDNA complexes (fewer than 40). Therefore, it is statistically insignificant to conclude about protein–ssDNA recognition vs. dsDNA recognition.

Experiments on monoclonal antibodies suggest that anti-dsDNA monoclonal autoantibodies exhibit preference for DNA binding motifs different from those of anti-ssDNA autoantibodies (Table 2).

Table 2  Similarities and differences between anti-ssDNA and anti-dsDNA (Literature compilation)

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>dsDNA binding</th>
<th>ssDNA binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG class</td>
<td>IgG and IgM</td>
<td>IgG and IgM</td>
</tr>
<tr>
<td>IgG subclass</td>
<td>IgG3</td>
<td>IgG3</td>
</tr>
<tr>
<td>Pathogenicity based on binding criteria</td>
<td>Some forms of lupus nephritis</td>
<td>Non-pathogenic?</td>
</tr>
<tr>
<td></td>
<td>CNS involvement</td>
<td>Pathogenic and nephritogenic in human and murine models</td>
</tr>
<tr>
<td></td>
<td>Correlates with disease activity</td>
<td></td>
</tr>
<tr>
<td>Abzymes-DNA hydrolytic activity</td>
<td>Human and mouse mono- and polyclonal</td>
<td>Mouse monoclonal.</td>
</tr>
<tr>
<td></td>
<td>Human? (Kats, 2008; Pavlovic et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Cytotoxic activity</td>
<td>Human polyclonal</td>
<td>Undetermined so far</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
On the basis of the observation that binding affinity decreases with increased number of base pairs in the DNA stem, a conformational selection binding mechanism is suggested where the Fab binds preferentially to unstructured state of the ligand (Tanner et al., 2001). More studies by Swanson et al. (1996) and Tanner et al. (2001) have confirmed high-affinity oligo-dT binding (Pavlovic et al., 2007).

Functions of hydrolysis and cytotoxicity: Computational data confirm theoretical predictions that hydrolysis of ssDNA being the substrate for abzyme. Extent of DNA in plasma greater than 100 ng/ml could be considered pathological. It is found that (Steinman, 1979; Gololobov et al., 1997) Fab fragments of monoclonal mouse anti-ssDNA antibody isotypes (IgG or IgM) show hydrolysis occurring three times faster than when the entire antibody molecule is analysed. Stronger expression of IgM and anti-ssDNA Fab fragments suggests the prediction that the location of enzymatic activity of the abzyme is probably the Fab fragment (shown via papain hydrolysis (Rodkey et al., 2000)). Whether the active site is comprised of light or heavy chain within the Fab fragment or a combination of both is an open question. However, underlying experiments suggest that both anti-ssDNA and anti-dsDNA could have hydrolytic activity and that this activity could be their pathogenic mechanism in autoimmune disease.

Isolated lupus human polyclonal anti-ssDNA autoantibody is capable of hydrolysing the substrates in a much slower manner than commercially available DNAse1 control (Kats, 2008). Relevant details (Figure 2) show a high level of purity at nanoscale and suggest that this feature is an intrinsic, constitutive activity of that particular antibody molecule.

**Figure 2** Electrophoretic analysis of the purity of anti-DNA antibody following a two-step affinity method employing magnetic beads (see online version for colours)

Pathogenicity: Antibodies that bind to hairpin-forming DNA ligands may be particularly prone to deposition. Tanner et al. (2001) have reported the first structure of a Fab complexed with hairpin-forming DNA. The ligand used for co-crystallisation is 5′-d [CTG (CCTT) CAG]-3′, predicting a hairpin structure with quadra-nucleotide loop (CCTT) and a stem of three base pairs.
6 Viral ds- and ss-DNA in autoimmune and other diseases

Possible candidates: There are two viral candidates namely, Parvovirus B19 (Lehmann et al., 2003.) and Epstein-Barr (E-B) virus (Harley and James, 2006) that trigger SLE and other autoimmune diseases with the production of anti-DNA antibodies. The structure of Parvovirus B19 analysed both biologically and computationally make the virus a viable candidate for induction of anti-ssDNA antibodies. The ssDNA of B19 virus is small about 5000 nt with a hair pin structure (stabilising the ss-chain) having palindromic sequences and five thymidine repeats. All such features make it suitable for antibody binding. There are clinical and experimental indications hypothesising that Parvovirus B19 could trigger lupus and other autoimmune diseases (Pavlovic et al., 2010) (Figure 3). Similar evidence exists for ds-DNA E-B, virus. With the location and transcription of EBV latent genes on the viral ds-DNA episome defined, it can be queried whether they induce production of anti-DNA antibodies.

Figure 3 Model of possible Parvovirus B19 association with autoimmunity:
(a) in sera: antimicrobial strategy and clearance of viral DNA apoptotic waste from sera by anti-ssDNA autoantibodies and (b) in cell nucleus: Nuclear DNA hydrolysis by anti-ssDNA nuclear autoantibodies with perpetuation and maintenance of ‘vicious cycle’ (see online version for colours)

Source: Pavlovic et al. (2010)

Studies indicate that microbial and viral infections are causative factors in autoimmune diseases (Pavlovic et al., 2010; Pisetsky, 2009; etc.). Posed questions thereof are

- Is the key antigen that causes autoimmune diseases is DNA?
- If so, should a vaccine be designed toward DNA?
- How might such a vaccine work?

Hydrolytic anti-DNA antibody instead of neutralising, actually maintains and perpetuates disease by constant DNA hydrolysis in the blood and cell nuclei. So, it is just needed to either shut down their secretion or block their activity. To stop secretion, the precise mechanisms of the secretion should be known (which are not, however, understood completely yet). To block the active site of the abzyme, the underlying coordination, activation and acceleration toward inducing hydrolysis should be first comprehended.

Thus far, human infection with B19 has not been prevented by vaccines based on structural viral candidates as antigen (Pavlovic et al., 2010). However, the seriousness of this disease calls for eliciting anti-ssDNA antibodies and for RVD. This virus has all of the characteristics of a substrate appropriate for binding anti-ssDNA antibody analysed
by Tanner et al. (2001), and it can be a viable candidate for similar binding of human lupus anti-ssDNA antibodies (Pavlovic et al., 2010). Variations in viral structures warrant special attention and specific approaches in RVD. Viral infection in dogs is seen successfully prevented by (a viral structural) protein-based vaccine. It leads to the question why humans cannot answer with the similar immune responses. Adjunct questions are

- Is viral DNA required as a component of the vaccine as primary or additional immunogen?
- And if so, what kind of immunological scenario can one imagine being produced in response to such a vaccine?

Unlike B19, E-B virus dsDNA has been shown to elicit production of anti-dsDNA antibodies. Site-directed mutagenesis experiments have shown that antibody can have two binding sites (Harley and James, 2006) one for ss- and the other for ds-DNA. Further studies are necessary to fully understand the meaning of such molecular interactions.

7 Methods for determination of ssDNA and dsDNA hydrolysis by anti-DNA antibodies

*State-of-the-art contributions:* The essential problem in trying to distinguish functional features and pathogenicity of ss- and dsDNA autoantibodies has been inadequate with non-specific methodology for their isolation and fine purification. Recent contribution (Pavlovic et al., 2007) based on two-step magnetic bead method to isolate and purify human autoantibody reactive with ssDNA is shown in Figure 2.

8 Computational approach as an aid in determining anti-DNA hydrolytic activity

*Induction of strain as the starting point:* Computer-assisted molecular design based on modelisation of chemical entities responsible for pharmacological activity and use of analytical models to describe the relationship between physicochemical properties and biological activities of such entities are necessary in vaccine design efforts (Banton et al., 2010). In general, the quality of molecular description with appropriate represent of the molecular interaction phenomenon decides the success of such non-wet-lab studies. Relevant approach via molecular interaction potential is more advantageous than other techniques in helping structure-activity studies.

9 Computational approach as an aid toward RVD

Advocating vaccination due to Edward Jenner (1789) has helped the humanity (Israeli et al., 2009) at large, and in spite of the success of global vaccination efforts, there exists still a niche for new and improved vaccines against numerous human pathogens. As such, RVD is sought to manipulate the immune system so as to ‘work harder’ through its cellular components facilitating antigen recognition aiming at
minimisation of trial-and-error approach of the current strategies
maximising the production of pathogen-specific immune cells in the body post-vaccination.

Possible steps in RVD are identification of a robust, cross-reactive neutralising antibody and its use as a template to identify the epitope that binds to it; reconstruction of the epitope (or its functional homologue) as an immunogen and determination of the most effective means to apply it.

In classical immunology approach, epitope discovery being of primary significance, epitope-prediction algorithms are used based on neural network libraries and matrix-based server (PROPERDI and so on). More flexible way can be on RVD-epitope identification via computational methods including structural and sequence analyses.

A typical RVD target is the Humoral Immune Response (HIR) facilitating virus elimination regulated by B-cells. Plasma B-cells secrete antibodies and memory B-cells recognise pathogens during re-infection. Analytical modelling help vaccine designs by providing insight into immune system functions such as the HIR. Such models formalise intuitive aspects of biological considerations in experimental results (Banton and Roth, 2010).

**Bioinformatics for epitopal candidates:** The vaccines that rely on epitopes (short antigenic determinants) are usually made of proteins recognised by the cellular arm of the immune system. In the case of epitopal candidates for RVD, structural analysis and epitope prediction servers are useful in epitope discovery in some viruses including Ebola virus (Banton et al., 2010). With analytical method applied to epitope extraction, epitope prediction servers can identify B- and T-cell epitopes. Further, sequences after the extraction can be tested in the PROSITE and OMA databases to identify protein fingerprints and patterns. Thus, the best viral protein candidate for vaccine and detection of new candidates (not previously detected by wet-lab methods) are ascertained saving time (over the wet-lab trial-and-error approach). Computationally-derived epitopes serving as templates for vaccine design can also explain viral evolution and protein conservation. Analysing more complex mechanisms and knowing components of general immune response toward RVD thereof for a variety of DNA viruses are open-platforms for future research.

**Bioinformatics for DNA vaccine candidates:** DNA vaccines are simple rings of DNA containing a gene encoding an antigen and a promoter/terminator inducing the gene expression in mammalian cells. For novel approaches in generating vaccines for all types of desired immunity with cytolytic T-lymphocytes, T-helper cells and antibodies, a technology for global usage is imminent in terms of manufacturing ease, broad population administration and safety. The basic scheme of contemporary DNA-based RVD is shown in Figure 4 illustrating the computational aid in RVD.

In view of infectious diseases for which conventional approaches have failed, (as with malaria, HIV and human Parvovirus B19), future vaccines might include only DNA vaccines and/or recombinant organisms (like vaccinia virus used as vaccine against smallpox) or their proteins. However, neither the protein components of the virus, nor the DNA would alone ‘work’ as expected.
DNA antigens can be poorly immunogenic, suffer from sequence variability, be difficult to present, express or purify as well as expensive to produce. The database of TIGR on microbial sequence information has been envisaged in bioinformatic computations; and some vaccines combining genome sequencing data, selection of subsets of immunologically relevant genes and in silico (computer) analyses have emerged in the post-genomic era. Relevant in vitro and in vivo testing for vaccine candidate’s identification is illustrated in Figure 5. Efforts on mining microbial DNA sequences and microbial gene-finding with the annotated information across 100 bacterial species (of TIGR and other labs) due to Glimmer 3, 2007 have led to Interpolated Markov Models (IMMs) in identifying coding regions and distinguish them from non-coding DNA.

Figure 5  Principles of Rational Vaccine Design
10 Summary and conclusions

More disease flares can be anticipated due to some autoimmune and lymphoproliferative diseases, DNA cleaving, anti-DNA antibodies being active. Stopping their production and whether immunising humans against their own antibodies are lingering questions. Though, the use of purified idiotypic anti-DNA antibody 16/16d being capable of preventing lupus in murine models of the disease has been suggested (Shoenfeld et al., 2002), such vaccination may be futile in humans indicating a need for a non-existing or non-discovered mechanism toward successful vaccination in humans.

The immunological role of anti-DNA hydrolytic antibodies via DNA neutralisation and clearance in autoimmune disease is abortive since they may not remove the immunogen-antigen (DNA). Hence, they have to be removed first so as to prevent the clearance of DNA from the body being affected. This cannot be done just by injecting any kind of DNA because that will elicit production of more antibodies, induce the flare, worsen the symptoms and decrease DNA clearance. In such situations, curing and prevention are distinguished with preventing the disease by designing a suitable vaccine emphasised. Knowing thereof the potential pitfalls of DNA vaccines and considering RVD as a possible and reliable tool are of current interest. Yet, there are several unanswered questions on the problem that require additional consideration as follows:

Size of DNA being disproportional to MHC molecules (that is, too large for APC-presentation) since the small pieces of 150–250-bp-DNA are excreted transrenally; location of hydrolytic active sites and causes of their activation, their peptide sequence and knowing the optimal epitopal candidate and comprehending the structural and conformational differences between non-hydrolytic and hydrolytic anti-DNA antibodies. In this context, the DNA itself has the potential to elicit the formation of anti-DNA hydrolysing and anti-DNA non-hydrolysing antibodies. The possibility of first precipitating the complexes via blood and tissue DNAses (clearance), instead of removing them altogether, could be a strategy in the relevant efforts on RVD previously described. However, this should be experimentally confirmed.

Though, autoimmune, hydrolytic, anti-DNA autoantibodies make complexes with DNA that precipitate under endothelial cells of small blood vessels (causing generalised vasculitis in SLE), the instant at which catalytic feature of anti-DNA abzyme is formed (over a 2-week evolution) is not known.

Relevant questions of interest are:

- How deep in the B-cell evolution, does the B-cell rearrangement process penetrate?
- If the gene-rearrangement is targeted, what happens in the immunoglobulin genes and when does it happen?
- What exactly is implicated vis-à-vis the error in the sequence or in re-combination during anti-DNA antibody production?
- Do they get modified from the beginning of their existence or wait during the development for an invader to activate their enzymatic capacity?
- Otherwise, do they undergo somatic mutations with time and/or experience the ‘stroke’ of some epigenetic phenomena (like glycosylation or chain elongation) during their post-translational modification?
If so, why does this happen in some individuals and not in others?

What category of B-cells does prevail?

Considering the observation of Diamond, 2005 that anti-DNA antibodies are secreted from both lupus reactive and non-reactive B-cells, how can such cells be fully marked and selectively separated to exercise the research on them?

Non-hydrolytic, natural anti-DNA antibodies do not have catalytic activity and they probably just neutralize the DNA molecule allowing the ordinary DNAses to hydrolyze the DNA molecule completely and remove it (clear it from the blood through glomerular filtration of uric acid as the final metabolic product of nucleic acids). This is desirable when viral or microbial DNA is high in the patient’s blood. The DNase kinetic properties differ from anti-DNA antibody’s (Pavlovic et al., 2010). The DNA quickly broken into small nucleotides by DNase and eventually excreted by glomerular filtration through the kidney will not precipitate as immune complexes on the basilar membrane. But, the DNA hydrolytic antibody kinetics break the DNA at a slower rate allows precipitating conditions for immune complex formation and deposition in the small blood vessels, including glomeruli in the kidney. Different kinetic parameters of these two considerations are discussed in Pavlovic et al. (2010) with suggestions thereof in Table 3.

Table 3  Kinetic parameters of DNase 1 and lupus anti-ssDNA antibody (Average of five ensemble measurements)

<table>
<thead>
<tr>
<th>Material</th>
<th>DNAse 1 (From bovine pancreas)</th>
<th>LP 4 lupus antibody</th>
</tr>
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<tbody>
<tr>
<td>( V_{\text{max}} )</td>
<td>188 pM/min</td>
<td>135 pM/min</td>
</tr>
<tr>
<td>( K_m )</td>
<td>94 pM</td>
<td>67 pM</td>
</tr>
<tr>
<td>Time</td>
<td>30–40 min</td>
<td>8 h</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Within the range of +22–54°C thermo-stable</th>
<th>Within the range of +4–37°C thermo-stable</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Activators: Divalent cations</th>
<th>Ca(^{2+}) not present</th>
<th>Ca(^{2+}) present at 0.5 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg(^{2+}) present at 10 mM</td>
<td>Mg(^{2+}) present at 5 mM</td>
<td></td>
</tr>
</tbody>
</table>

DNases over a stretch of time bind instantly, specifically, competitively and firmly, (Pavlovic et al., 2010) and renders partially under the appropriate conditions the precipitation. Furthermore, DNA concentration being high in the blood, the DNases are transient, degrading rapidly, so that antibodies (produced at a faster rate) have a higher chance of binding to the substrate in an appropriate ratio and hydrolysing it. As for the cells, the situation is similar since different DNases present in cells do the clearance during regular DNA repair processes. However, anti-DNA antibodies can penetrate living cells and hydrolyse DNA in the cell nucleus. What DNA may encounter is another question except that both ds- and ss-viruses use the host replication mechanisms to reproduce their own DNA.
Thus, the anti-DNA antibody theoretically once in the nucleus has the opportunity to attack viral ds- or ssDNA, and the host’s own unmethylated DNA content increases concurrently. This suggests that lupus in children may be triggered by microbial mechanisms. Suppose unmethylation starts exceptionally early (for some reason) in some children. No relevant information at this time is yet available.

Naturally occurring, non-catalytic antibodies just neutralise and separate or mark the excess of DNA of any origin in the blood, while other mechanisms later destroy it completely (probably DNAses from the blood and tissues). Thus, it might still be considered as a physiological mechanism of removal of excessive DNA from the blood. Yet, it is opined that anti-DNA antibodies with non-catalytic activity present in some individuals can become catalytic and induce clinical development of autoimmune disease at some critical point. But the reasons and details of such process are lacking. Hence, whether a vaccine should be created to prevent autoimmune diseases and, if so, how this can be done consistent with the role of catalytic antibodies in RVD can be outlined as follows:

- Antigen–protein produces antibody against catalytic antibody (global and DNA-specific) corresponding to the catalytic part of hydrolytic anti-DNA antibody. This requires defining the location and sequence of a particular self-anti-DNA (ds- or ss-) catalytic centre defining the epitope through bioinformatics procedures. Wet-lab studies can check T-cell priming and B-cell activation and production of specific antibodies against the catalytic site. This may theoretically block hydrolysis if injected. Bioinformatics analysis can help defining the activation centre and optimise the choice of prospective epitopal candidates. Wet-lab data are necessary to confirm active centre location and develop the vaccine. Whether a complete vaccine (with some adjuvant) is realised is for a future study to work on.

- Viral DNA (ss- or ds-) as antigen omits antigen presentation to T-cells and keeps the DNA features within B-memory cells (primed ex vivo with DNA). Use of computer data and bioinformatics can decide the suitable approach. It involves exploring the DNA library for the best ss- and ds-viral candidates. Here, RVD can also help to target the best possible scenario. The immune system is then trained to produce anti-DNA antibodies from B-cells that memorise the DNA and binding there by just neutralising the antigen. This should produce anti-DNA non-hydrolytic antibodies in higher concentration to ‘demarcate’ the known DNA (memorised) and allow (other already mentioned and known) mechanisms to eliminate it from the circulatory system of the body.

Design, production and optimisation of useful catalytic antibodies can benefit from computer-aided analyses and predictions through bioinformatic formulations. It is possible to produce different models of antibodies and predict the optimal outcome of their catalysis before entering the wet lab. Such antibodies are already in use for preventive (HIV-1 vaccine) and curative (cancer treatment) purposes.

The occurrence of anti-DNA antibodies (both non-catalytic and catalytic) is still not fully understood. As mentioned before, despite a lot of theoretical consideration, the aspects of B-cell secreting either anti-ss or dsDNA antibodies are not comprehended. Therefore, it is not easy to prevent or cure diseases that are consequents of anti-DNA hydrolytic antibodies. Extensive studies on immunogenic mechanisms of different DNAs (human, mammalian, microbial and viral) are necessary to contribute more knowledge.
and solve the problem. Precise sorting of antigen-presentable DNA candidates must be critically considered. Studies of DNA-sensing protein mechanisms in B-memory cells should be enhanced to determine whether they transduce DNA-image sequences to B-memory cell responsive elements. Computational approach, though has not been exhaustively deployed, can viably shorten the time-consuming, wet-lab, trial-and-error experimentations orchestrating more straightforward designs with optimised features of candidates for vaccines.

In spite of identifying immunological scenarios in different autoimmune diseases, there is not yet a clear idea on efficient treatment and/or eradication of disease mainly due to the reason that the cause is not known. With clinical description for example, on lupus being a disease “with a 1000 faces”, individual ways of prevention and therapy of diseases are open questions.

In a recent study, combining the use of short CpG oligonucleotides with Anthrax Vaccine Absorbed elicited de novo production of protective antibodies by high-affinity memory B-cells (Klinman et al., 2009). This finding establishes a novel benefit of CpG oligonucleotide adjuvants and has implications in assessing the efficacy of future anthrax and other vaccines. Further, B-cell memory population expansion indicates that T-cell memory is not the only cellular system on which the immune system relies on in remembering an antigen due to cytosolic activity in DNA-recognising proteins in B-cells (Muruve et al., 2008). Relevant result suggests the relationship between DNA recognition, intracellular TLR activation and B-cell memory induction with secretion of protective antibodies (Pavlovic et al., 2010). Small proteins found in the genome of Ebola (Banton et al., 2010) and other viruses may also induce anti-DNA antibody formation. No study is yet attempted to detect such proteins in the genome of diseases other than autoimmune diseases. They could also be a potential source of DNA-mimicking antigens.

**Figure 6** Possibilities for RVD in diseases linked to anti-DNA antibodies’ secretion

RVD and relevant links toward vaccination against autoimmune diseases (with secretion of hydrolytic anti-DNA antibodies)

Conceptual steps of vaccine design (with hydrolytic anti-DNA antibodies)

- Protein-based vaccine
  - Modeling
  - Location of active sites
  - Coordination of active sites
  - Acceleration of active sites
  - Definition of epitopal part of active sites (for targeting therapy or vaccination)
  - Modeling of molecules for blocking the active site, or in vivo immunisation
  - Synthesis of blocking molecule, or optimising antibody concentration for applications
  - Using idiotypic anti-DNA antibodies to entire Ab and/or active sites and testing for adjuvants

- DNA-based vaccine
  - Finding out the part of DNA immunogenic (via DNA fingerprints from blood and white blood cells)
  - Defining the best possible candidate
  - Synthesising oligo
  - Checking whether B-long-memory cells are produced
  - Optimising the vaccine *via in vitro* wet-lab experiments
  - Injecting oligo with follow ups *in vivo*
  - Seeking for adjuvant if necessary and using computational approach to ascertain the best candidate
The summary on the relationships and molecular interplay of antigens and antibodies within the spectrum of anti-DNA hydrolytic antibody production and secretion is visualised in Figure 4, and Figure 6 is self-suggestive on the roles of RVD indicating RVD trials in autoimmune diseases being hallmarked by anti-DNA hydrolytic antibodies. Hence, it can be concluded that anti-DNA hydrolytic autoantibodies can be a link toward new anti-(DNA) RVD. More collaborative wet-lab and bioinformatics research is needed to support this conclusion assertively. Relevant studies on mouse are encouraging (Blank and Shoenfeld, 2008).

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References


From Pauling’s abzyme concept to the new era


