Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Structural basis for the function of anti-idiotypic antibody in immune memory

J. Vani<sup>a,d</sup>, Jhinuk Chatterjee<sup>b</sup>, M.S. Shaila<sup>a</sup>, R. Nayak<sup>a,c</sup>, Nagasuma R. Chandra<sup>b</sup>,<sup>*,</sup>

<sup>a</sup>Department of Microbiology and Cell biology, Indian Institute of Science, C.V. Raman Avenue, Bangalore 560 012, Karnataka, India
<sup>b</sup>Bioinformatics Centre, Indian Institute of Science, C.V. Raman Avenue, Bangalore 560 012, Karnataka, India
<sup>c</sup>National Institute of Science Education and Research, Bhubaneswar, India
<sup>d</sup>Centre de Recherche des Cordeliers, Equipe 16 - Immunopathology and Therapeutic Immunointervention, University Pierre et Marie Curie - Paris 6, UMR S 872, Paris F-75006, France

**Abstract**

We had earlier proposed a hypothesis to explain the mechanism of perpetuation of immunological memory based on the operation of idiotypic network in the complete absence of antigen. Experimental evidences were provided for memory maintenance through anti-idiotypic antibody (Ab<sub>2</sub>) carrying the internal image of the antigen. In the present work, we describe a structural basis for such memory perpetuation by molecular modeling and structural analysis studies. A three-dimensional model of Ab<sub>2</sub> was generated and the structure of the antigenic site on the hemagglutinin protein H of Rinderpest virus was modeled using the structural template of hemagglutinin protein of Measles virus. Our results show that a large portion of heavy chain containing the CDR regions of Ab<sub>2</sub> resembles the domain of the hemagglutinin housing the epitope regions. The similarity demonstrates that an internal image of the H antigen is formed in Ab<sub>2</sub>, which provides a structural basis for functional mimicry demonstrated earlier. This work brings out the importance of the structural similarity between a domain of hemagglutinin protein to that of its corresponding Ab<sub>2</sub>. It provides evidence that Ab<sub>2</sub> is indeed capable of functioning as surrogate antigen and provides support to earlier proposed relay hypothesis which has provided a mechanism for the maintenance of immunological memory.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Antibodies specific for determinants within the variable region of an antibody molecule are known as anti-idiotypic antibodies. Anti-idiotypic antibodies produced against the combining site idiotope may mimic the original antigen and are said to bear an "internal image" of the antigen. Jerne’s network theory (Jerne, 1974) predicts that idiotypic and anti-idiotypic interactions constitute an immune network that is involved in the regulation of the immune responses. The immune system has been shown to be a functional idiotypic network and anti-idiotypic antibodies have been shown to be components of the normal immune system (Gilles and Remy, 1994).

The ability of the immune system to ‘remember’ a previous encounter with an antigen is the hallmark of the adaptive immune response. Immunological memory forms the basis for prophylactic vaccination and is generally believed to be maintained by long living memory cells (Zinkernagel et al., 1996). Many aspects of immunological memory are still poorly understood. Recently, even the very existence of immunological memory has been questioned and the memory phenomenon is attributed to pre-existing neutralizing antibodies and activated T cells (Zinkernagel and Hengartner, 2006). However, the mechanisms which keep the neutralizing antibodies and pre-activated T cells at a reasonable level in the body to be effective long after primary infection or vaccination are not yet well defined.

We had earlier proposed a hypothesis to explain the mechanism of immunological memory (Nayak et al., 2001) by way of mutual interaction between complementary idiotypic and anti-idiotypic B cells through the idiotypic determinants in the variable region of the antibody specific for antigen. The anti-idiotypic cells carry a mimic of the antigen which drives memory response further by triggering idiotypic memory T and B cells. A role for serum immunoglobulins in the perpetuation of immunological memory has also been proposed (Nayak et al., 2005).

We have provided experimental support to this theory by the demonstration that idiotypic and anti-idiotypic B cells are generated in the same animal after immunization with antigen (Mitra-Kaushik et al., 2001). Recently we have presented evidence for antigen-specific B and T cell responses elicited by either anti-idiotypic antibody (Ab<sub>2</sub>) immunization or anti-id variable region DNA specific immunization. We have also shown that Ab<sub>2</sub> variable region derived peptides similar to the T cell epitopes of the antigen can mimic the antigen with respect to MHC binding and induction of T cell immune responses providing proof for the presence of
processed and presented peptidomics in the system after antigen immunization (Vani et al., 2007a,b,c).

Although structural information on idiotypic and anti-idiotypic antibodies against viral (Ban et al., 1994), tumor antigens (Luo et al., 2000; Chang et al., 2005) and allergen antigens (Hantusch et al., 2006) are available, the implication of structural mimicry between antigen and Ab2 that is responsible for antigen specific immune and memory response has not been described. In this work, we present a structural basis for functional mimicry shown by anti-id in maintenance of immune memory. We have used molecular modeling of antigen and Ab2 as well as their complexes and report computational analysis that provide insights into structural mimicry of antigen recognition by Ab2 molecules.

We have chosen hemagglutinin protein of Rinderpest virus as a model antigen. Rinderpest virus, belonging to morbillivirus family of Paramyxoviridae, is antigenically closely related to Measles virus. H protein is a protective antigen of the virus and is one of the envelope glycoproteins. We have previously produced and characterized an anti-idiotypic monoclonal antibody (Ab2) D9D8 (Vani et al., 2007a) generated using a monoclonal antibody A12A9 (Ab1) specific for H protein (Mitra-Kaushik et al., 2001). D9D8 behaves as an ‘internal image’ of H protein as shown by its ability to induce anti-idiotypic antibodies (Ab2) in mice and rats (Vani et al., 2007a). It has also been shown to mimic H protein in terms of both B and T cell responses (Vani et al., 2007a) suggesting that besides the functional significance have been mapped using monoclonal antibodies (Renukaradhya et al., 2002) generated by using the Smith and Waterman local alignment algorithm (Smith and Waterman, 1981) as implemented in the GCG software suite. The energy minimized models of the H and Ab2 were subjected to standard structural validation checks by using Pro-check to ensure the geometric and steric correctness of the models.

3. Results

3.1. Molecular models of H and Ab2

The H protein sequence, when scanned against NR blast (http://www.ncbi.nlm.nih.gov) using Blosum-62 substitution matrix, revealed significant (~60%) similarity with several other hemagglutinins in the database (Fig. 1A), of which the highest similarity was seen with the equivalent proteins from other Paramyxoviruses, the closest of them being the H protein of Measles virus. The crystal structure is available in PDB (PDB ID: 2ZB5) and it was used as the template to build the model of Rinderpest virus H protein ecto domain (Fig. 1B). B cell epitopes and neutralizing epitopes on H protein have been mapped previously (Renukaradhya et al., 2002; Sugiyama et al., 2002). 2.2. Homology modeling of H antigen

The deduced amino acid sequence of the hemagglutinin protein H of Rinderpest virus vaccine strain (RBOK) was taken from the Genbank (accession number: CA83182). The heavy and light chain variable region sequences of monoclonal antibody (Ab2), D9D8 specific for H protein were earlier determined (Vani et al., 2007a) and the sequence has been deposited in the Genbank (accession number: AY523599).

2.2. Homology modeling of H antigen

The deduced amino acid sequence of the hemagglutinin protein of Rinderpest virus (RPV-H) consisting of 608 amino acids, was subjected to sequence analysis using Blast (Altschul et al., 1990) against sequences of proteins in PDB, to identify possible structural templates. The closest similarity observed was with Measles virus H protein (MV-H), for which a crystal structure (PDB ID: 2ZB5) is available (Hashiguchi et al., 2007).

Model building was carried out using Swiss modeler (Arnold et al., 2006). The lengths of the template and the H sequences were comparable without any significant insertions or deletions. The ecto domain of Rinderpest virus H (192–608 aa) encompasses experimentally mapped B and T cell epitopes (Renukaradhya et al., 2002).

2.3. Homology modeling of anti-idiotypic antibody (Ab2)

The sequence of variable region of Ab2 is a 104-aa long heavy chain and 92 aa long light chain (Vani et al., 2007a). Each chain was analyzed both individually as well as a single unit to identify suitable structural templates in the Protein Data Bank, using Blast (Altschul et al., 1990). The identified structural templates (templates chosen for heavy chain: 1IFH, 1BLN, 1IGT, 1QLE, 1AD1, 1QXT, 1UB6, 32C2 and the templates chosen for light chain: 32C2, 1IFH, 1BLN, 1J05, 1IQW) were further superposed onto a common framework and the differences among them were studied in order to find the most optimal combination of H and L chain templates. Subsequently, a common template 32C2 was chosen and the two chains were built and energy minimized using standard homology modeling protocols using Insight-II and CNS (Brunger et al., 1998). The method used is similar in that used by WAM (Martin et al., 1989), a well accepted protocol for antibody modeling. The preliminary models thus obtained were subjected to rigid body minimization followed by simulated annealing using CNS software suite.

The sequences of H and V_H-V_L chains of Ab2 were aligned in different combinations in order to identify any regions on the Ab2 molecule that may bear similarity to the previously identified epitope region on H recognizing mAb1. Sequence alignments were carried out by using the Smith and Waterman local alignment algorithm (Smith and Waterman, 1981) as implemented in the GCG software suite. The energy minimized models of the H and Ab2 were subjected to standard structural validation checks by using Pro-check to ensure the geometric and steric correctness of the models.
Fig. 1. (A) Alignment of sequences of several morbillivirus H proteins (see Section 2). Histograms indicate the level of similarity between the conserved regions. (B) 3D model of β-propeller domain of Rinderpest virus H viewed along the quasi-six fold axis (top view). Secondary structure elements in the ribbon diagram are colored blue to red from the N to the C terminus. The epitope recognized by the Ab\textsubscript{1} (A527-L556) is shown in violet color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
surface was analyzed in particular. Based on this analysis, 32C2 (structure of an activity suppressing Fab fragment to cytochrome p450 aromatase) was chosen as the final template (Fig. 2B).

The built model of Fv of the Ab2 is shown in Fig. 3A. The model of Ab2 based on 32C2 when subjected to rigid body minimization, that resulted in a model that with an angle of about 129° between the H and the L chains.

### 3.2. H protein epitopic loop has similar structure as that of CDR loops of Ab2

The epitope for Ab1 on the H protein has earlier been shown to map to the region between 527 and 556 aa (Mitra-Kaushik et al., 2001). On subjecting the complete sequence of H protein to secondary structure prediction analysis, the mapped 30 aa epitopic region was found to consist of 2 anti-parallel beta strands, connected by a loop.

The sequence alignments in Fig. 4 showed that epitope of H aligned with CDRs of both VH and VL of Ab2. Previously it has been shown that peptides synthesized from these region (from H as well as Ab2) have the ability to function as both B and T cell epitopes (Vani et al., 2007c). Structural superposition of Ab2 VH with that of epitopic loop on H in Fig. 3B shows significant similarity in these segments, matching with the sequence alignments. Of the experimentally mapped 30 residue epitope of H, residues 539–556 aa aligns with 49–65 aa residues of CDR2 in the H chain. A weaker but still comprehensible similarity of a portion of H with VL is also observed. This is not a surprise, as the light and heavy chains of the antibody having the same structural fold. The observed similarities are despite the fact that H and Ab2 molecules on the whole adopt different structural folds belonging to different SCOP classes and are also of different sizes serving as a beautiful example for generating functional mimicry through sub-structural similarity.
Fig. 3. (A) Molecular model of D9D8: Fv region is shown in grey color, while the pink colored loops depict the V\textsubscript{H} CDRs and the cyan colored regions represent the V\textsubscript{L} CDRs. (B) Superposition of Ab\textsubscript{2} heavy chain (yellow) with a segment of hemagglutinin protein (Blue). Red colored regions depict the 551–554 aa region of hemagglutinin epitope and green colored region depict the 51–54 aa region of Ab\textsubscript{2} heavy chain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 4. A schematic representation of different homologous regions between epitope on H protein and VH and VL of Ab\textsubscript{2}: regions of sequence similarity are colored (Vani et al., 2007a). T cell epitopes as predicted by several MHC-peptide binding prediction algorithms (Vani et al., 2007c) are shown. B cell epitopes were analyzed by antigenicity plot. The antigenicity index was more than +1 for all the marked regions, as computed with a Jameson–Wolf plot (Jameson and Wolf, 1988). It is significant that the region of structural equivalence also matches with these regions.

4. Discussion

Anti-idiotypic antibodies are potent immuno regulators which can either enhance or suppress the expression of idiotypic specificity (Lopez-Requena et al., 2007). The combining site of these anti-idiotypic antibodies not only mimics the original antigen at the level of primary or secondary structure, forming an internal image of the antigen (Garcia et al., 1992) but also able to exhibit a functional activity which mimics the physiological activity of the antigen (Taub and Greene, 1992). Very few studies have attempted to determine the mechanisms underlying the antigen specific response that certain anti-idiotypes can elicit.

In our previous work, we had shown that the idiotypic and anti-idiotypic B cells are generated in the same animal after immunization with antigen (Mitra-Kaushik et al., 2001) and immunization of syngeneic mice with antigen or idiotypic antibody generates idiotype and antigen-specific T cells (Mitra-Kaushik et al., 2002). We have recently shown that peptidomimics in the CDRs of anti-idiotypic B cells, which may not be completely homologous to the original antigen peptide but which carry the structural complementarities, are able to trigger idiotypic antigen-specific T cells (Vani et al., 2007c). Further, we have shown that antigen-specific T and B cell responses are elicited on immunization with anti-idiotypic antibody in the absence of antigen and also that boosting with antigen-specific anti-idiotypic B cells generates memory response in antigen-primed mice (Vani et al., 2007a).

The present work was undertaken to evaluate if structural similarity between antigen and anti-id antibody, could provide a basis for functional mimicry. The molecular modeling results suggest that there is indeed structural mimicry which may complement the functional activity of anti-idiotypic antibody, especially in its membrane bound form. We have demonstrated the presence of structural homology between the monoclonal murine anti-idiotypic antibody and antigen by identifying the cross-reactive residues responsible for mimicry. Identification of threshold level at which B cells provide lasting memory as well as the synergy between T cell and B cell responses would assist our understanding of the development of long-lasting immune memory. The sequences on the epitopic sites of H and regions on V\textsubscript{H} of Ab\textsubscript{2} are shown in Fig. 4 to depict functional as well as structurally equivalent parts of the molecules taking part in elicitation of immune memory. The Fv fragment of Ab\textsubscript{2} exhibits similar antigenicity values as that of the region on H protein carrying Ab\textsubscript{1} epitope.
The isolation of human recombinant anti-idiotypic scFv against coronavirus from a non-immune phage display library has been reported (Lamarre and Talbot, 1997). However, these scFv antibodies were not able to induce an antiviral immune response sufficiently strong to protect immunized animals. The anti-id for H protein is an Ab2β signifying that it carries the internal image of the antigen and the present work has shown that structural mimicry between the H protein and Fv may be the key element in induction of anti-H response in vivo, mediated by Ab2.

From the present analysis, several conclusions can be drawn on the existence of anti-ids in the repertoire of an individual. The anti-idiotypic Fabs generated can act as true internal images of the antigen and the present work has shown that structural mimicry between the H protein and Fv may be the key element in induction of anti-H response in vivo, mediated by Ab2.

Acknowledgements
The use of computational facilities at the Bioinformatics Centre, Interactive Graphics Facility and Super Computer Education and Research Centre at the Indian Institute of Science are gratefully acknowledged.

Funding: The infrastructure support provided by the Department of Biotechnology, Government of India, under program support on Basic Biology of Microbial Pathogens is gratefully acknowledged.

R.N. is an emeritus medical scientist of Indian Council of Medical Research.

References
Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Arnold, K., Bordoli, L., Kopp, J., Schwede, T., 2006. The SWISS-MODEL workspace: a web-based environment for protein structure homology modeling. Bioinformatics 22, 195–201.

Ban, N., Escobar, C., Garcia, R., Hasel, K., Day, J., et al., 1994. Crystal structure of an idiotypic–anti-idiotypic Fab complex. Proc. Natl. Acad. Sci. U.S.A. 91, 1604–1608.

Brunger, A.T., Adams, P.D., Clore, G.M., DeLano, W.L., Gros, P., et al., 1998. Crystallography & NMR system: a new software suite for macromolecular structure determination. Acta Crystallogr. 54D, 905–921.

Chang, C.C., Hernandez-Guzman, F.G., Luo, W., Wang, X., Ferrone, S., Ghosh, D., 2005. Structural basis of antigen mimicry in a clinically relevant melanoma antigen system. J. Biol. Chem. 280, 41546–41552.

Garcia, K.C., Desiderio, S.V., Ronco, P.M., Verroust, P.J., Amzel, L.M., 1992. Three-dimensional structure of an angiotensin II-Fab complex at 3A: hormone recognition by an anti-idiotypic antibody. Science 257, 502–507.

Gilles, J.G., Remy, J.S., 1994. Healthy subjects produce both anti-factor VIII and specific anti-idiotypic antibodies. J. Clin. Invest. 94, 1496–1505.