bNAber: database of broadly neutralizing HIV antibodies

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ABSTRACT

The discovery of broadly neutralizing antibodies (bNAbs) has provided an enormous impetus to the HIV vaccine research and to entire immunology. The bNAber database at http://bNAber.org provides open, user-friendly access to detailed data on the rapidly growing list of HIV bNAbs, including neutralization profiles, sequences and three-dimensional structures (when available). It also provides an extensive list of visualization and analysis tools, such as heatmaps to analyse neutralization data as well as structure and sequence viewers to correlate bNAbs properties with structural and sequence features of individual antibodies. The goal of the bNAber database is to enable researchers in this field to easily compare and analyse available information on bNAbs thereby supporting efforts to design an effective vaccine for HIV/AIDS. The bNAber database not only provides easy access to data that currently is scattered in the Supplementary Materials sections of individual papers, but also contributes to the development of general standards of data that have to be presented with the discovery of new bNAbs and a universal mechanism of how such data can be shared.

INTRODUCTION

Broadly neutralizing antibodies (bNAbs) neutralize multiple viral strains, in contrast to non-cross-reactive antibodies that are specific for individual strains. Their discovery changed our views of how humans can deal with quickly mutating viruses, such as HIV, influenza and hepatitis C, and is one of the most exciting discoveries in immunology in the last several years. Among other new discoveries, bNAbs have opened new avenues in the quest for the development of a vaccine against HIV/AIDS, as bNAbs against antigens such as HIV envelope glycoprotein (Env) can be used as templates for the design of vaccines (1). Following on this promise, the number of groups working on identifying new HIV bNAbs and the number of known antibodies started to grow rapidly. Several large studies now in development guarantee even faster growth in incoming years. However, at this point, detailed information on newly identified bNAbs is available only in supplementary materials of individual papers, with no common resource collecting all available information on HIV bNAbs and no general standards about what set of data should be presented for each new antibody.

For example, both the number and clade composition of viral strains used to calculate neutralization data (IC50 and IC80) and even the precise definition of when an antibody can be called broadly neutralizing vary from study to study (2). Different assay protocols or different virus panels can give different results. For one antibody (2F5), different studies reported the breadth of neutralization being between 39% and 67%, as different studies used different neutralization panels (3–5). Compounding these inconsistencies is the fact that there is no single resource that collects information about HIV specific bNAbs. Some data are available from the IEDB-3D epitope database (6) and LANL’s HIV Molecular Immunology Databases (7), via the ‘Neutralizing Antibody Resources’ page in the Immunology section at http://www.hiv.lanl.gov/. IEDB-3D provides data on HIV antibodies with experimentally determined structures but has no data about neutralization breadth and efficiency and, more importantly, it does not present any information on antibodies without experimental structures (6).
The LANL resource offers a ‘Summary of Best Neutralizing Antibodies’ table with links to papers, Ab sequences and structures, notes on breadth of neutralization, and references to the tables and figures in original publication, as well as list of Ab contacts or key residues. Actual neutralization data, however, are not available, making it difficult for the neutralization profiles of different antibodies to be compared, and difficult to perform any kind of comparative analysis of bNAbs without collecting needed information from primary literature. Also, neither IEDB-3D nor LANL’s ‘Neutralizing Antibody Resources’ have mechanisms for submitting data on new bNAbs.

bNAber (short for broadly Neutralizing Antibodies electronic resource) provides access to raw data on broadly neutralizing HIV antibodies, including sequences, structures and neutralization IC50 data, as well as in-house and third party software to analyse it. Its ultimate goal is to support immunogen design for the development of an HIV/AIDS vaccine. Although bNAber database is primarily addressed to AIDS research community, we expect that the general importance of the bNAb field will entice interest from much broader group of researchers. bNAber is freely available at http://bNAber.org and does not require any login or registration.

DATA INTEGRATION AND CURATION

Two types of HIV can be distinguished genetically and antigenically: HIV-1 is the cause of the current worldwide pandemic, whereas HIV-2, found mostly in West Africa, is less easily transmitted and is not considered a worldwide health risk. More than 90% of HIV/AIDS cases are caused by infection with HIV-1 viruses group M, the most common type of HIV (8). The M group is subdivided further into clades, called subtypes that are also given a letter.

The HIV neutralization is currently tested in the TZM-bl/pseudovirus assay (5). This assay measures neutralization in TZM-bl cells as a function of a reduction in Tat-induced luciferase reporter gene expression after a single round of virus infection. There is no standard accepted percent cutoff used for bNAbs definition, but most of bNAbs described in literature neutralize at least 30% of strains in large TZM-bl panels (more than 100 HIV strains). The neutralization breadth is defined as % of strains neutralized by this Ab. The median antibody concentrations required to inhibit HIV activity by 50% (IC50) is referred to as neutralization ‘potency’ or ‘depth’.

The bNAber database schema was developed by combining fields from data tables in Walker et al. (2) with fields used in LANL table ‘Summary of Best Neutralizing Antibodies’, additional fields were suggested by the database beta testers from the Scripps CHAVI-ID groups. Data on a set of currently known 60 HIV bNAbs were downloaded from the LANL HIV, PDB and GenBank databases and converted into bNAber database tables. The tables were further carefully curated with data manually extracted from individual papers, listed in the Supplementary On-line Materials.

Raw data on neutralization were parsed from supplementary materials of manuscripts describing individual bNAbs and loaded into the database. A continuously updated list of papers used as a source of data for bNAber are provided as part of the Online-only Supplementary Data. All imported data were carefully curated prior to loading into the database, including fixing erroneous or non-standard bNAb and virus strain names. An automated pipeline will be developed to ease the database updates.

WALK THROUGH THE DATABASE AND TOOLS

The bNAber home page provides an interface to access all of the database content. There, a user can select individual antibodies or groups of them for further analysis by different criteria including donor, study, neutralization breadth (% of strains neutralized with IC50 or IC80 < 50 µg/ml) and depth (median IC50 (µg/ml) against viruses neutralized with an IC50 < 50 µg/ml). Selection by epitope can be done both from a drop-down menu and from a graphical interface of the HIV spike protein model. After selection, clicking the ‘submit’ button opens up a summary page of selected antibodies and a drop-down menu allowing the user to select further actions. On the summary page, clicking on underlined names of specific antibodies opens individual pages with full information about each antibody in the database (see Figure 1 for a sample series of steps through the main pages of the database). The antibody details page combines data on neutralization efficiency (median IC50 (µg/ml) against viruses neutralized with an IC50 < 50 µg/ml) from all available studies. In addition, links with PDB codes of coordinates (when available) provide access to an integrated Jmol (9) structure viewer, which allows researchers to view complex structures, including precalculated antibody–antigen binding sites (epitope on antigen and paratope on Ab). This functionality is discussed in greater detail in the Online Supplementary Data (Supplementary Figures S1–S3).

Five actions highlighted in the pull-down menu from the summary page are available in the current bNAber release, others are in development and the date of their availability is indicated on the menu. Three of the actions, ‘Show sequences and download FASTA file’, ‘Percent viruses neutralized with an IC50 < 50 µg/ml, per clade’ and ‘Median IC50 (µg/ml) against viruses neutralized with an IC50 < 50 µg/ml, per clade’ are simple and self-explanatory. The third one, ‘Overlay structures’, links to a POSA server (10) for multiple structure analysis, which is discussed below. The action ‘Show aligned sequences’ leads to the page which presents aligned Abs amino acid sequences and allows a user to perform further sequence analysis by tools such as Jalview (11). Using Jalview, a user can color sequence alignments, build phylogenetic trees and correlate Abs sequence similarity with their neutralizing properties (e.g., by comparing trees with clustered heatmaps obtained from the next tab, as described below).

In addition, navigation tabs on the home page provide access to other features and functionalities of the database.
Neutralization heatmaps

This tab provides access to an interface for clustering of the neutralization data based on the similarity of neutralization IC50 values against individual virus strains. Heatmaps are popular tools to present matrices with large amount of data in a convenient, compact form. Heatmaps of hierarchically clustered data can be used to assess similarity and differences of objects (Abs and virus strains in our case), to find the number of distinct groups of objects, and to correlate the grouping of objects with their other properties. This tool can help a user answer questions such as comparing neutralization data for several Abs to determine differences and similarities in their behavior relative to particular virus clade or strain, comparing neutralization data obtained in different studies, or compare and correlate grouping of bNAbS based on neutralization properties and on sequence similarity, epitope or other features. Neutralization data can be presented in two forms: numerical colored table, such as found in papers describing neutralization data, and heatmaps. Browsing large neutralization tables require scrolling through the page, but a heatmap view of neutralization table allows large datasets to be easily viewed on one screen (Figure 2). Hierarchical clustering in turn helps researchers show groups, similarities and differences in the neutralization data of several antibodies tested on large panels of HIV strains (Figure 2). The heatmap is generated using the Euclidean distance and average-linkage clustering method and the log10 values of the IC50 neutralization data for a subset of virus strains (utilizing an IC50 of 50\(\mu\)g/ml for values designated as >50\(\mu\)g/ml) and bNAbS that are displayed on the axes.

POSA multiple structure comparisons

This tab brings the user to the POSA server (10) page with precalculated results of structure alignments of all pairs of HIV bNAb structures and an interface to perform further analysis. The POSA server for multiple protein structure alignment by partial order graphs was developed previously in our group and provides several tools and interfaces to study structural similarity and diversity between homologous proteins (10). The superposition is driven by the structural alignment of selected chains—it can be an antigen or Heavy or Light chain of an antibody. At the next step, other chains are superimposed using the same transformation matrix and root mean square deviation is calculated for all equivalent C-alpha atoms. This approach can identify even subtle differences in the binding mode of the antibody and the antigen. The interface presents the aligned sequences and structures in multiple windows, making it easy for users to analyse and compare the structures of antibody–antigen complexes. Independently, access to the precalculated results and to the POSA interface is available from a pull-down menu on the summary pages available from the home page followed by manual selection of specific coordinate sets (see the Discussion section below). Pairs and groups (up to five) of antibodies can be selected and their multiple structure alignment can be analysed by the POSA visual interface. Structure comparisons are...
presented using a novel, multi-window display of protein complexes. Figure 3 shows an example of POSA structure comparisons of bNAbs complexes PDB:2NY7 and PDB:3U7Y aligned on the antigen structure.

Submit new bNAb

This tab provides an interface for easy input of data (Supplementary Figure S4, see Supplementary Data) on a new bNAb. The suggested list of features is based on the recently published bNAb discovery papers and LANL table ‘Summary of Best Neutralizing Antibodies’. For large-scale neutralization studies we invite researchers to submit data tables (through the provided interface), which we will upload into the database. Before submission, the form will be validated for required fields as well as for expected values (e.g., neutralization data should be non-negative). Once the form has been validated, the submitted data are put into a temporary holding area, which is periodically validated by the annotator. Once the annotator and the bNAb team have validated that the data are a genuine scientific contribution, the data are sanitized against MySQL injection attacks and inserted into the database, at which point the new data are automatically displayed on the bNAb web application.

Biologist view

bNAber provides multiple menu driven interfaces and an option to submit user-edited SQL queries (in development). However, we anticipate that many users will have limited experience in using on-line databases and in this tab we provide access to several queries defined by specific user questions. Clicking on a specific question on the list brings the users to a dedicated query designed to answer this question. We have polled the beta testers of the bNAber database about questions they would like to have answered. bNAber team invites users to provide us with additional questions.

Help, use cases and database download

Access to general help, including links to several precalculated examples, database tutorial and on-line Supplementary Material are provided in this tab. ‘Use cases’ section has step-by-step instructions on how to perform several typical database tasks, including

![Figure 2. Heatmap neutralization data for selected bNAbs (IC50 in µg/ml) shown in alphabetical order (top panel) and ordered by hierarchical bi-clustering of the IC50 neutralization data (bottom panel) for the same subset of virus strains (log10 of IC50 data were used, with IC50 >50 µg/ml replaced by 50). See On-line Supplementary Data for references to tools used to create this image.](image-url)
Figure 3. POSA structural superposition of two antibody-antigen complexes, 3U7Y (NIH45-46 Fab in complex with gp120 of strain 93TH057 HIV-1) and 2NY7 (Broadly Neutralizing CD4-Binding-Site Antibody b12 and gp120) with primary alignment on the antigen gp120 chains. The superposition illustrates the difference between the two antibodies bound to the same epitope, but at different angles relative to the gp120 surface. Separate windows below allow for separate viewing of individual antibodies.
comparing median IC50 neutralization data of two bNAbs in different studies. Additionally, an option to export the bNAbs database is available here. An option to download specific tables and data for subsets of bNAbs is also available on some pages.

DISCUSSION

The ultimate goal of the bNAber database is to support immunogen design for HIV/AIDS vaccine development by providing easy access to data on all known HIV bNAbs and facilitating their analysis. Understanding the relation between molecular data on neutralizing Abs, Env and structure of Ab–Env complexes should facilitate the generation of antigens presenting protective epitopes (1). By developing bNAber we aimed to integrate publicly available, but dispersed data on HIV bNAbs into a single database with a unified interface. bNAber provides detailed information on all known HIV bNAbs, including raw neutralization data from TZM-bl assay, sequences of bNAbs, when available, activity against alanine mutants, predicted epitopes, and 3D structures of bNAbs and their complexes with antigens. Neutralizing activity of bNAbs against pseudovirus panels is used to compute median IC50 and percent of viruses neutralized for full panels or their subsets, thus opening the way to compare data from different labs or panels.

Data in bNAber are available using a series of user-friendly interfaces designed to support typical search strategies. Users have multiple ways of selecting antibodies for comparative analysis, including study, recognition site (gp160 epitope), donor and breadth or depth of neutralization (high or low). Presenting data for a selected group of bNAbs provide a convenient way to understand the antibody properties important for immunogen design. Multiple analysis types applicable to the selected groups of bNAbs, including multiple sequence and pairwise structure alignments, neutralization heatmaps and others are available from the pull-down menus. The 3D information, including precalculated structure comparisons of bNAbs, is presented using a novel, multi-window display of protein complexes. This display provides researchers with a convenient way to explore the binding modes of the various antibodies and compare different conformations of the same antibody.

bNAber differs significantly from other HIV antibody databases. IEDB is an epitope-centric resource and contains information on anti-HIV antibodies which structures are available in PDB in complexes with antigens. bNAber focuses on multiple data types on broadly neutralizing HIV Ab and provides information on Ab without structures as well. At the same time bNAber has less structures then IEDB because it concentrates only on bNAbs. Examples of HIV antibodies that were not considered for inclusion in the resource: N5-i5 Fab (3TNN), 13H11 Fab fragment (3MO1, 3MNW, etc.) or N12-i15 (3QEH, 3TMN, 3TNN), bNAber in comparison with LANL’s HIV Molecular Immunology Database provides access to bNABs aligned sequences, structures, raw neutralization data and integrated analysis tools.

The information in the database is currently extracted manually from literature, including supplemental material, and additional information is downloaded from the LANL HIV Molecular Immunology Database, PDB and GenBank. We believe that the basic data structure in the bNAber database can serve as a prototype for a standard set of data that minimally should be submitted when new bNAbs are discovered. Therefore, we ultimately intend to rely on community data depositions, especially depositions accompanying paper publications. To this end, we provide a separate interface for outside users to submit data, either entered manually or uploaded to the database.

We will work with the AIDS community and NIH to develop a mechanism for direct deposition of data on new bNAbs accompanying new publications to bNAber. Such mechanism may eventually replace the current system of providing information on bNAbs in supplementary data, which uses different standards and is generally not compatible between different journals.

The bNAber database was created for the users who want to: (i) analyse in details sequence, 3D structure, neutralization profiles, escape mutations, etc. of anti-HIV Abs; (ii) design, synthesize and express genes coding new immunogens aiming to elicit bNAbs; (iii) analyse 3D features of broad neutralization; (iv) sequence antibody genes in immunized animal/patient and track the development of the Ab genes leading to broad neutralization; and (v) correlate features of sequence and structure of Abs with their ability to cross-neutralize. The resource could be of interest to researchers developing vaccines to other diseases, including influenza, hepatitis C, dengue and West Nile viruses (12).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online, including [2,13–23].

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