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How germinal are human antibodies ?

An attempt to assess the human nature of engineered antibodies

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ABSTRACT :

Antibody affinity maturation depends on somatic hypermutations but the percentage of identity between expressed human IgGs, and the corresponding germline V and J genes from which they derive, has never been extensively quantified. Knowledge of such naturally occurring percentage is of fundamental interest, and it would allow to define the human "nature" of an antibody.

Defining the human "nature" of an antibody, a notion that can be distinguished from its human "origin", is of great interest as only the former notion is central to tolerance. Importance of this notion is indeed best examplified by chimerization and humanization processes that apply to antibodies of non-human origin in order to give them a "human nature". In contrast, *in vitro* mutagenesis used to improve affinities of antibodies, in particular those originating from human naïve libraries, tends to diminish their human nature. Use of non-human primate antibodies for therapeutics (see for instance Chassagne *et al*, 2004; Laffly *et al*, 2005) also depends upon their ability to be regarded as being of (or close to) human nature.

Here, using IMGT® definitions and databases (http://imgt.cines.fr), we have calculated the percentage of identity (called here "germinality") between expressed IgGs and the sequences coded by the germline V and J genes from which they derive.

MATERIAL AND METHODS :

Utilising IMGT® tools (in particular the IMGT/V-QUEST software (Giudicelli *et al, 2004*) used as a sub-routine), IMGT® databases, FR-IMGT and CDR-IMGT definitions (Lefranc *et al, 2003*), a program was designed to automatically determine the germline V and J genes from which any human IgG is derived. The program then translates the sequences and calculates the percentage of identity between the expressed sequences and the corresponding germline V and J genes.

The results (or «germinality », expressed as a percentage) are calculated for each FR-IMGT and CDR-IMGT, but it should be noted that only the V-encoded part of CDR3 (or 3'V-REGION) is analysed. Our goal is to calculate the mean germinality for each FR-IMGT or CDR-IMGT of γ, κ and λ chains of human IgGs .

In order to better verify the IgG nature of the analysed sequences, understand the potential causes for variation in the germinality and as a quality control, it was decided to only include, in our study, the sequences referred to in published articles. It appeared that litterature references attached to the sequences, in the databases, are often inaccurate so that exact references often had to be found manually. This information, regarding human IgGs, will later be included in IMGT® databases.

As the influence of antigens (for instance, polysaccharides) or individual donors on affinity maturation and thus germinality is not well known, and also as a quality control, it was decided to use statistical studies (ANOVA) to determine if a germinality estimate can be regarded as average, before including it in the general mean calculation. Homogeneous groups of sequences (same conditions of affinity maturation as defined by immunization and donor, same isotype) had to comprise at least seven sequences to be analysed by this parametric test before being included in the calculation. Immune libraries or hybridoma sequences were included in the study, but not naïve libraries, as they contain Fab or scFv fragments of IgM origin. In the case of γ chains, sequences obtained after priming in γ CH1 regions, with no other indication of origin, were also included.

Here, as a **preliminary study**, IgG sequences originating from three normal non immunized donors, one patient with lupus (De Widt *et al, 1999*; Varade *et al, 1993*), and donors immunized with protective antigen *(B. anthracis)* (Wild *et al, 2003*), Puumala virus (Salonen *et al, 1998*), capsular polysaccharide of *S. pneumonia* (Zhou *et al, 2002*), rhesus antigen (Chang *et al, 1998*), and one patient with Bloom's syndrome (that affects a DNA helicase) (Sack *et al, 1998*) were analysed. They represented 472 γ, 288 κ and 168 λ chains **(928 sequences as a whole).**

RESULTS :

Germinality (%)	FR1	CDR1	FR2	CDR2	FR3	CDR3 (V coded)	FR4	Nb of analysed sequences (see above)	Nb of sequences included in the mean calculation (see below)
γ chains	93.2	85.1	93.7	81.9	90.7	89. 3	95.2	428	247
κ chains	92.8	87.6	95.9	97.3	96.5	79.8	96.8	288	145
λ chains	91.8	88.1	91.7	90.6	95.4	72.4	95.1	168	136
Average	**92.6**	**86.9**	**93.8**	**89.9**	**94.2**	**80.5**	**95.7**	294.7	176

There is no significant difference neither between germinality of all FRs (as estimated for each isotype) nor between germinality of CDR 1 and 2, except for CRD2 of κ chains. **The calculated mean germinality value is 93.5 % for FRs and 88.4 % for CDR1 and 2. These values are close**, as best examplified by CRD2 of κ chains whose germinality is not significantly different than for κ FRs. This observation is in agreement with the fact that most of diversity, and of interaction energy, is often regarded as being due to the CDR3 (of which only the V-encoded portion is analysed here) (see Bedouelle *et al*, FEBS, in press, for an example).

The γ and κ chains of patients immunized by polysaccharides and of one of the non-immune donor were shown by ANOVA to have lower germinality values than all the other groups, so that they were excluded from the mean germinality calculation. This lower germinality could be due to more affinity maturation by mutagenesis than average (more particularly by donors vaccinated by *S. pneumonia* polysaccharide) or to errors due to primers (many differences with germline sequences were located at the sequence extremities, for one of the non-immune patient). The λ chains of the patient bearing a lupus were shown by ANOVA to have a higher germinality than the other groups and were excluded from the rest of the study. However, as the γ and κ chains originating from this patient with lupus were not significantly different from the average, it cannot be excluded that this λ chain difference is not meaningful.

PERSPECTIVES :

Autoimmune patients and normal donors immunized with polysaccharides will be excluded from future work, which will focus on establishing normal mean germinality values with **all sequences, available in databanks, that follow our criteria**. Analysis of IgGs originating from autoimmune patients will be regarded as a separate work, as will be the analysis of the **entire CDR3**.

The mean germinality values could be used to assess the human nature of any given (including engineered) antibody.

This work is slowed by inaccuracy of litterature references attached to sequences in databanks, and authors are respectfully invited to check them after article acceptance.