P-009 COMPARATIVE ANALYSIS OF THE CDR-H3 REGION OF ANTIBODIES IN DIFFERENT HUMAN B CELLS AND PLASMA CELLS SUBSETS

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Introduction

The diversity of Ig molecules is initially generated by recombination of different germline gene segments (V, D, J segments) during B cells development. The antigen-binding sites of Ig molecules are mainly formed by six hypervariable loops termed complementary determining regions (CDR): three from the light (L) chain and three from the heavy (H) chain. CDR1 and CDR2 are the regions where somatic hypermutations (SHM) mainly occurs. CDR-H3 is directly formed by the juxtaposition of V-D-J regions and is located at the center of the antigen-binding site having then a key role in antigen recognition and binding. At first, antigen-induced PCs generated in inductive lymphoid tissues are selected to travel through the circulation and finally, are selected to home onto terminal deposit organs. Previously our group has explored the mutational modifications occurring during the systemic PC maturation, by comparing the SHM of IGHV genes (specially, in CDR1 and in CDR2 regions) in human PCs isolated from tonsil, blood, and bone marrow (BM). The SHM analysis revealed the existence of a maturational gradient in these genes, as demonstrated by a progressive increase in the frequency of total and R mutations and total and non-conserved amino-acids changes following the direction: tonsil→blood→BM.

Objectives

The aim of this work is to study the CDR-H3 composition differences in the previously studied human PC subsets (tonsil→blood→BM) and compare between them as well as with other subsets, ie naïve B cells, memory B cells and intestinal lamina propria (LP-PCs).

Materials and Methods

All sequences were obtained from our previous works and other from published data bases. Sequences were analyzed submitting them to the ImMunoGeneTics (IMGT/V QUEST) web-based analysis tool. Data analysis were then imported into a Microsoft® Excel® based software, named Immunoglobulin Analysis Tool (IgAT) for further analysis. We analyzed a total of 1844 productive rearrangements: B cells (n=167); Memory B cells, (n=293) and CPs, (n=1384).

Results

As expected, the total number of mutations (R+S) and R/S ratio, was getting higher in the direction from B cells, memory B cells, tonsil-PC, blood-PCs, BM-PCs and LP-PCs the largest. Regarding to the CDR3 region, the number of mutations was similar between B cells and memory B cells, but was higher in the population of PCs. No exceptional differences between the uses of D-segments were observed between all subset compared. However, its length was lower in all the PCs populations compared to B cells and memory B cells, probably by use of shorter D-, J-regions and a reduced number of "N"-insertions. Moreover, the frequency of the amino acids and hydrophobicity index found in CDR-H3 regions varies from B-cell to PCs subsets.

Conclusions

These findings suggest that, as was described about somatic hypermutation in IGVH region, exist a maturational gradient from B cell to PCs which could be reflected in terms of CDR-H3 length and amino acid composition.