The use of bioinformatics to investigate the association between Hepatitis C and Rheumatoid Factor in the pathogenesis of Mixed Cryoglobulinaemia

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Introduction and Aims

Autoimmune conditions and immune-related renal disease may be triggered by infections including exogenous viruses or Human Endogenous Retroviruses (HERV) (ref 1,2,3).

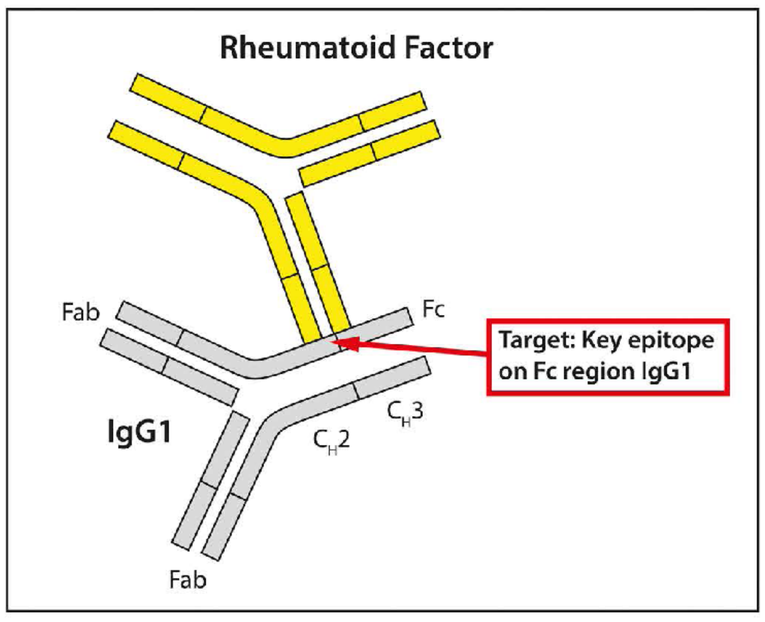
- The pathogenesis of Type II mixed cryoglobulinaemia is poorly understood, but is associated with hepatitis C virus (HCV) infection in up to 90% of cases.
- Circulating cryoglobulins contain polyclonal IgG and a monoclonal IgM Rheumatoid Factor, directed against IgG.
- Rheumatoid Factor binds to the Fc region of IgG1 (Figure 1)
- Renal biopsies in mixed cryoglobulinaemia show subendothelial immune complex deposits which contain IgM, and the presence of HCV antigens in immune complexes has also been reported.
- We have previously used bioinformatic computer programmes to demonstrate homology between the amino acid sequences of HERV-K10 matrix protein and IgG, that is recognised by Rheumatoid Factor (ref 1,2), and between HERV HRES-1 and some lupus autoantigens (ref 1, 3).

Bioinformatic analysis and molecular modeling was employed to investigate whether molecular mimicry might explain the association of HCV and Rheumatoid Factor in the pathogenesis of Type II mixed cryoglobulinaemia .

Methods

- Amino acid sequences for HCV proteins and IgG1Fc were obtained from the NCBI/Brookhaven database.
- We investigated whether molecular mimicry can be demonstrated between HCV protein and the target for Rheumatoid Factors on IgG1Fc by bioinformatic software LALIGN.
- Molecular modeling was undertaken using 2 software packages PyMOL and UCSF Chimera.
- The position of amino acid sequences in IgG1Fc that showed molecular mimicry between HCV and IgG1Fc were compared with the position of predicated Rheumatoid factor epitopes and immunodominant amino acids, which are likely to be antigenic sites (ref 4)
- Since it has been suggested that cryoglobulins have an affinity for fibronectin, bioinformatic analysis between predicted Rheumatoid Factor epitopes and fibronectin was undertaken.

Figure 1
Rheumatoid Factor Binds to Fc region of IgG1



Results

Bioinformatic analysis of Hepatitis C (Accession no. ABO38658) and IgG1Fc (Accession no. AF150959) revealed 3 potential regions of amino acid homology with IgG1Fc, 5 or 6 amino acids in length and with 1 or 2 amino acid substitutions. (Figure 2)

Figure 2
Homologies between HCV and IgG1Fc

IgG1Fc sequence (Accession No. AF150959.1)
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GPFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

HCV	FNWAV	PPLLES	PPIPPP
IgG1Fc	FNWYV	PPVLDS	PPCPAP

- A 4 amino acid sequence is sufficient to be an antigenic site and the chance of a 4 amino acid homology occurring purely by chance is 1:160,000.
- Amino acid substitutions had high positive replacement scores, suggesting little effect on peptide conformation.
- All 3 peptides were located in the C_H2 or C_H3 domains of IgG1Fc
- Molecular modeling showed the single amino acid substitution in FNWAV produced only minor change of peptide conformation (Figure 3)
- The FNWAV sequence was located the C_H2 domain and was fully within a known epitope site for Rheumatoid Factor antibodies in (Figure 4), and is likely to be a potential molecular mimic.
- PPLLES conformation was also little changed (Figure 3), was partly within a Rheumatoid Factor epitope area in C_H3 domain, but was fully in an antigenic area (Figure 4).
- In contrast, with the PPIPPP sequence, at the N terminus of the CH2 domain, amino acid substitutions significantly changed molecular conformation (Figure 3), and it was only adjacent to an antigenic site (Figure 4).
- Homology between predicted Rheumatoid Factor epitopes and fibronectin showed a number of alignments, including 100% homology of the sequence QQGN.

Figure 3
Molecular mimicry between Hepatitis C and IgG1Fc

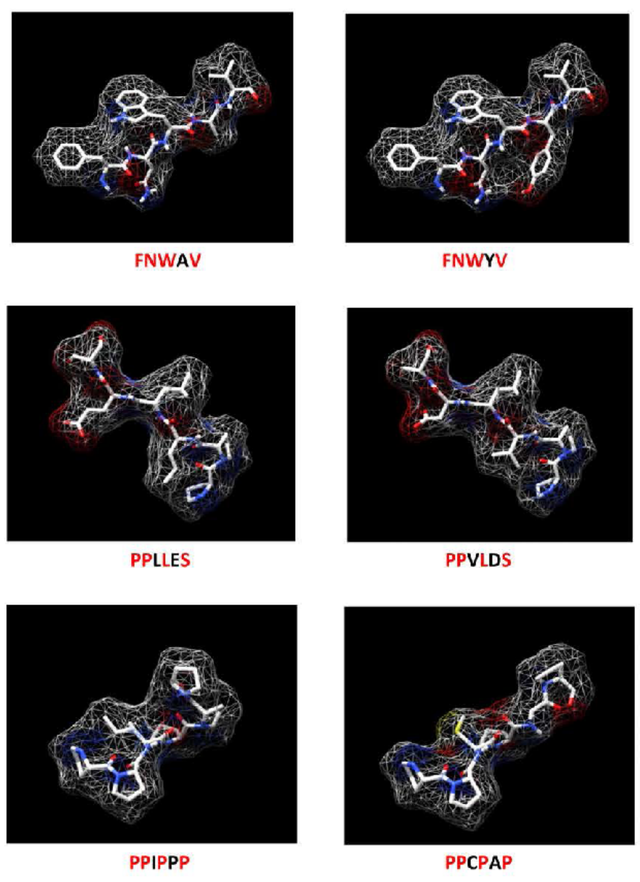


Figure 4 - Location of homology of FNWYV and PPVLDS in the C_H2 and C_H3 domains of IgG1Fc

IgG1Fc - Homology with HCV and Rheumatoid Factor epitopes

TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGPF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IgG1Fc C_H2 domain - Homology with HCV and location of Immunodominant amino acids (i.e. potential antigenic sites)

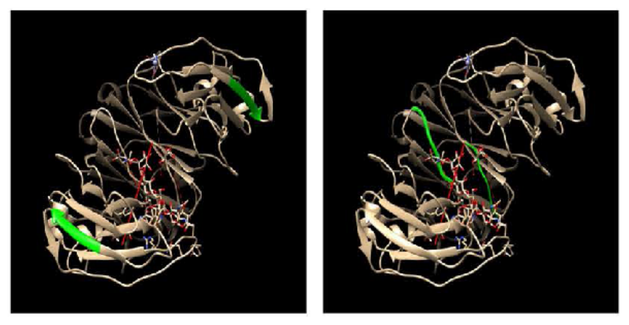
TCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

IgG1Fc C_H3 domain

GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FNWYV in C_H2 domain

PPVLDS in C_H3 domain



Locations of FNWYV and PPVLDS in IgG1Fc A and B chains are indicated by green arrows

Conclusions and Discussion

- Molecular mimicry between HCV and the target for Rheumatoid Factor on IgG1Fc has been demonstrated.
- This gives the potential for these cross-reacting antigenic determinants being involved in the pathogenesis of Type II mixed cryoglobulinaemia.
- The proposal is reinforced by the regions of homology being in the domains of known antigenic sites on C_H2 and C_H3 region of IgG1Fc.
- The significance of molecular mimicry between Rheumatoid Factor and fibronectin needs to be further investigated.
- It is postulated that key HCV epitopes, as molecular mimics of Rheumatoid factor, might also bind to IgG1Fc, and could contribute to the pathogenesis of cryoglobulins and immune complexes.
- It needs to be determined to whether patients with mixed cryoglobulinaemia, and also rheumatoid arthritis, have alterations in amino acid sequences in the IgG1Fc region that predispose to the binding of Rheumatoid Factor.
- These results give the possibility of the development of therapeutic synthetic blocking peptides or antibodies.
- This experimental approach could be useful in investigating other immune-related renal diseases.

References

1. Tugnet, N, Rylance, P, Roden, D., Trela, M., Nelson, P Human endogenous retroviruses (HERVs) and autoimmune rheumatic disease: Is there a link? The Open Rheumatology Journal, 2013; 7: 13-21
2. Nelson, P.N., Roden, D., Nevill, A., Freimanis, G.L., Trela, M., Ejtehadi, H.D., Bowman, S., Axford, J., Veitch, A.M., Tugnet, N., Rylance, P.B. Rheumatoid Arthritis is associated with IgG antibodies to Human Endogenous Retrovirus HERV-K10 Gag Matrix: a potential pathogenic mechanism of disease? J. Rheumatology 2014; 41:1952-60
3. Nelson, P., Rylance,P., Roden, D., Trela, M., Tugnet, N. Viruses as potential pathogenic agents in systemic lupus erythematosus Lupus, 2014; 23: 596-605
4. Nelson,P.N., Westwood, O. M.R., Freimanis, G., Roden, D., Sissaoui, S., Rylance, P., Hay, F.C. Comparison of Antigenic Regions Identified on igGFc Using Bioinformatics vs Pepscan Analysis Clinical Medicine: Arthritis and Musculoskeletal Disorders 2008; 1: 1-10

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