CR011

Structural and functional differences between human and non-human cell expressed human TNF RII-Fc Chimera

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INTRODUCTION

Most proteins undergo post-translational modification (PTM), which can alter their physical and chemical properties, e.g., MW, pI, folding, stability, and biological activity.

Glycosylation is the most widely found PTM, with estimations that 80% of all plasma proteins are glycosylated.

Glycosylation is not under direct genetic control, but is dependent on the availability and activity of the various glycosyltransferases, monosaccharides and precursors.

Glycosylation of recombinant proteins is dependent on the machinery of the cell line in which they are made.

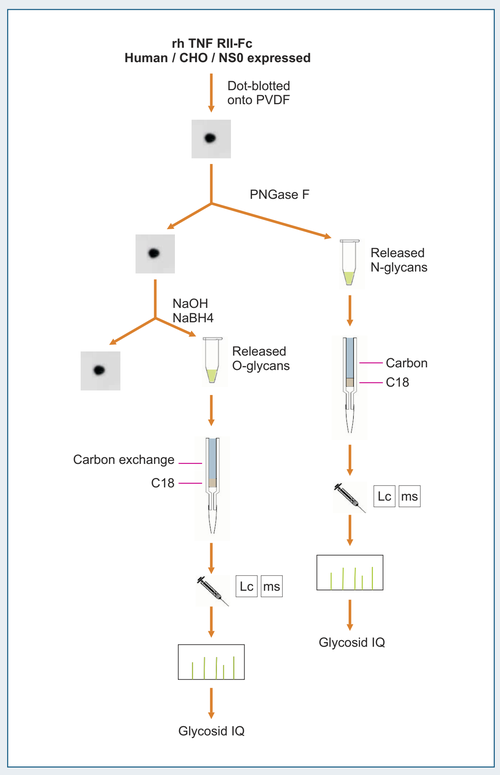
Thus, human recombinant proteins expressed in different cell lines have different glycosylation.

Apollo Cytokine Research produces human recombinant Receptor II fused to IgG1 Fc (TNF RII–Fc Chimera) expressed in human cells (modified 293).

Here we present the differences in glycosylation and antigenicity of Apollo's TNF RII-Fchcx to other commercially available forms produced by expression in CHO and NS0 cells.

METHODS

GLYCAN ANALYSIS



ANTIGENICITY TESTING

Two groups of outbred Swiss mice (10 per group) were vaccinated 2 times with rh TNF RII-Fchcx and rh CHO TNF RII-Fc administered sc in FIA on days 0 and 28. Serum was taken on day 42 and the antibody response to both proteins measured by ELISA. Data from 1:16 dilution of serum is represented in the graph.

RESULTS: N-GLYCAN ANALYSIS

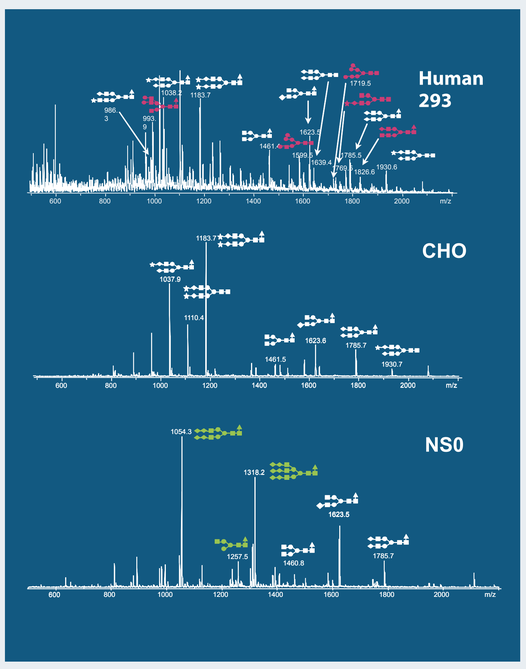
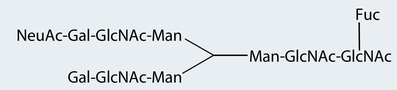


Fig 1. *MS of N-glycans released from rh human, CHO and NS0 expressed TNF RII–Fc*

Composition	MW	%		
		hcx	CHO	NSO
Hex3, HexNAc3, Fuc	1259			4.7
Hex3, HexNAc4, Fuc	1462	3.2	4.1	3.8
Hex4, HexNAc4, Fuc	1624	8.4	8.3	18.2
Hex4, HexNAc4, NeuAc	1769	4.3		
Hex4, HexNAc5, Fuc	1827	3.1		
Hex4, HexNAc5, NeuAc	1972	1.8		
Hex5, HexNAc4	1640	2.4		
Hex5, HexNAc4, Fuc	1786	11.3	7.8	13.2
Hex5, HexNAc4, NeuAc	1931	6.3	10	
Hex5, HexNAc4, Fuc, NeuAc	2077	23.7	25.2	
Hex5, HexNAc4, NeuAc2	2222		12.8	
Hex5, HexNAc4, Fuc, NeuAc2	2368	2.5	30.9	
Hex5, HexNAc5, Fuc	1989	18.6		
Hex6, HexNAc3	1599	2.9		
Hex7, HexNAc4, Fuc	2110			32.5
Hex7, HexNAc6	2370	9.3		
Hex8, HexNAc2	1720	2.3		
Hex9, HexNAc5, Fuc	2637			23

The major N-glycan structures in rh TNF RII-Fchcx were found to be:



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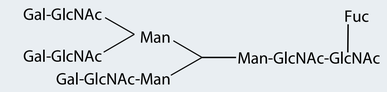
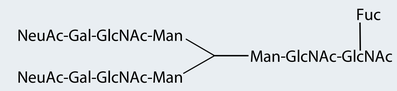


In comparison, the major N-glycans on rh TNF RII-Fc expressed in CHO cells were:



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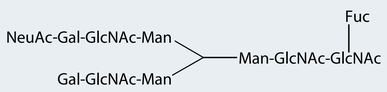
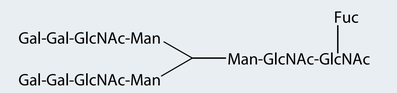


While, the major N-glycans on rh NS0 TNF RII-Fc protein were:



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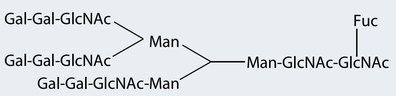


RESULTS: O-GLYCAN ANALYSIS

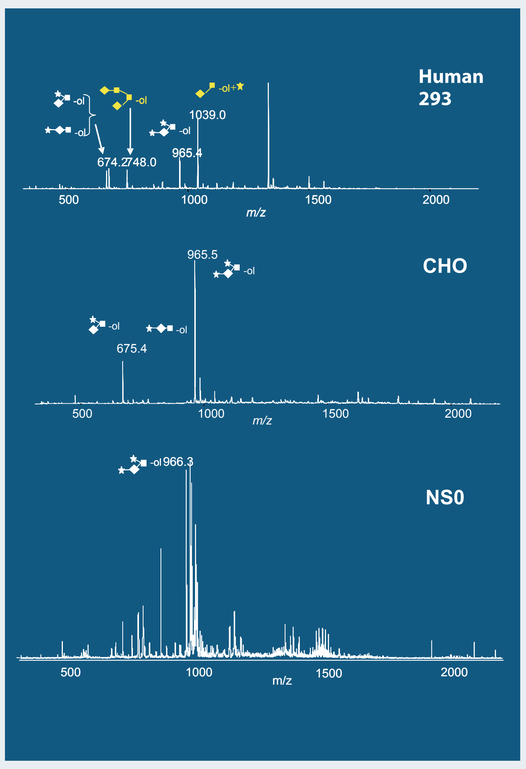
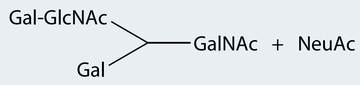


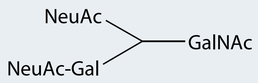
Fig 2. *MS of O-glycans released from rh human, CHO and NS0 expressed TNF RII–Fc*

Composition	MW	%		
		hcx	CHO	NSO
Hex, HexNAc, NeuAc	674	7	29	
Hex, HexNAc, NeuAc2	965	11	71	100
Hex2, HexNAc2	748	5		
Hex2, HexNAc2, NeuAc	1039	30		
Hex2, HexNAc2, NeuAc2	1330	47		

The major O-glycans in the rh TNF RII-Fchcx protein had extended type 2 core structures:



By comparison, the major O-glycan in CHO and NS0 expressed rh TNF RII-Fc proteins were the disialylated core type 1 structure:



SUMMARY OF GLYCAN RESULTS

- rh TNF RII-Fchcx contained mainly complex N-glycan structures including some triantennary and tetraantennary structures.
- rh CHO TNF RII-Fc contained only complex N-linked biantennary structures, no triantennary or tetraantennary structures were found.
- Sialic acid in rh CHO TNF RII-Fc was found to be exclusively alpha 2-3 linked, whereas the rh TNF RII-Fchcx protein had nearly equal amounts of alpha 2-6 and 2-3 linked sialic acid (results not shown).
- Major N-glycans on the NS0 expressed rh TNF RII-Fc were complex biantennary with teminal Gal-Gal extensions. No sialylated N-glycan structures were found.
- O-linked glycans in the rh TNF RII-Fchcx were more diverse than those found in both the CHO and NS0 expressed protein.

RESULTS: ANTIGENICITY TESTING

The antibody response elicited to and against both antigens showed that firstly the response elicited to the CHO cell material was higher than that to human expressed material ($P<0.01$). Additionally the response elicited by either antigen was higher when tested against the CHO material than to the human expressed protein ($P<0.01$).

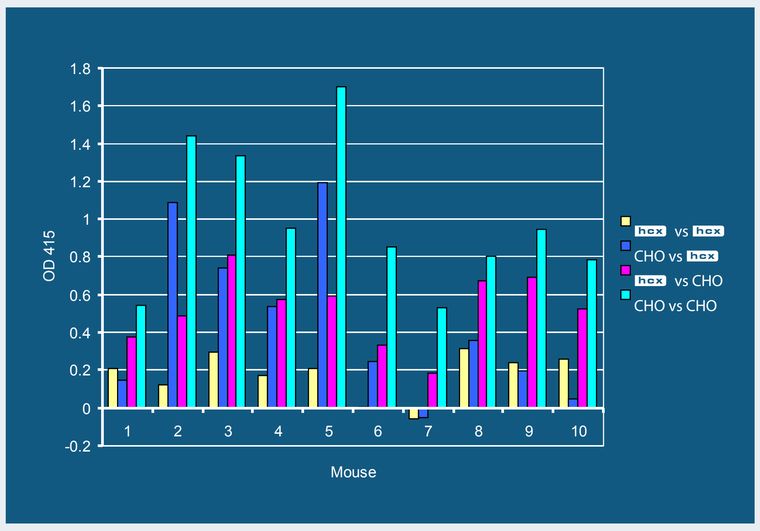


Fig 3. *Ab Response to rh TNF RII-Fchcx and rh CHO TNF RII-Fc*

CONCLUSION

The data observed demonstrates that the CHO expressed material is more immunogenic in mice than human expressed material. Also the antigenic epitopes appear to be more exposed and available for recognition on the CHO expressed material than when the TNF RII-Fc was expressed in human cells.

This data is complementary to the glycan analysis and suggests that human cell expressed material would be more preferable for clinical use than CHO expressed TNF RII-Fc.