

Does endogenous glycosylation prevent the use of mouse monoclonal antibodies as cancer therapeutics?

Carl A.K. Borrebaeck, Ann-Christin Malmborg
and Mats Ohlin

Monoclonal antibodies have many potential therapeutic benefits. However, when applied to humans, mouse monoclonal antibodies have several disadvantages. Here Carl Borrebaeck and colleagues describe a strategy to overcome the anti-Gal activity, thought to be one of the reasons why mouse mAbs have a limited half-life.

Mouse monoclonal antibodies (mAbs) have been used as therapeutics in a large array of clinical trials in the fight against cancer and infectious disease¹. The overall effect of these agents has in general been disappointing, especially in the extended treatment modalities often used for cancer (for review see Refs 2–4). The explanations for this include poor antibody specificities, low antibody affinity and a host anti-mouse antibody response (HAMA). However, the circulation half-life for mouse monoclonal antibodies has always been mysteriously short, in the range of 19–42 h (Refs 5 and 6), compared to 21 days for human IgG. In most studies, a simultaneous appearance of a HAMA response has not been obvious, which would have been the logical explanation, but such a response is normally not detectable until after 5–6 days after injection⁶.

Recently, Galili and co-workers described the presence of naturally occurring anti-Gal α 1-3Gal antibodies in the human population and in old world monkeys (Refs 7–9 and *Immunol. Today*, this issue), but not in other mammalian species. It was suggested that this natural antibody titer was triggered by foreign substances, such as infectious agents, dietary components, etc. This natural anti-Gal activity was estimated to amount to 1% of the serum γ -globulins in humans, which corresponds to approximately 100 μ g anti-Gal IgG ml⁻¹ human serum⁸. We propose that the short serum

half-life and thus the poor clinical efficacy of mouse monoclonal antibodies is related to an endogenous Gal α 1-3Gal glycosylation of these molecules¹⁰. To test this hypothesis, a number of mouse mAbs were tested for their reactivity with affinity purified anti-Gal antibodies isolated from normal human serum. As can be seen in Table 1, the majority of the antibodies contained Gal α 1-3Gal residues and it is worth noting that several of the clinically most relevant antibodies, for example 17-1A, 19-9 and HMFG-1 were particularly heavily glycosylated. The endogenous glycosylation found on mouse mAbs will immediately result in an immunocomplex formation when these antibodies are introduced into the circulation of human patients. In contrast, human mAbs produced from Epstein-Barr virus (EBV) infected human peripheral blood mononuclear cells do not show any signs of Gal α 1-3Gal-containing residues (Table 1). Furthermore, human mAbs derived from hybridomas formed using a human-mouse heteromyeloma fusion partner did not, except in one case, react with the human anti-Gal antibodies. One human mAb against digoxin (MO6 in Table 1) showed a positive reac-

tion, indicating the presence of Gal α 1-3Gal-residues on that particular antibody. This might indicate that the use of heteromyelomas for the production of human mAbs in some cases might lead to an unwanted endogenous glycosylation, similar to that found on the mouse mAbs.

The immunological barrier formed by the human anti-Gal antibodies efficiently prevents the use of xenografts in human immunotherapy since the graft will immediately be opsonized and killed by antibody-dependent cellular cytotoxicity (ADCC)¹¹. In relation to antibody-dependent immunotherapy, the results presented here offer an explanation as to why mouse mAbs have performed inadequately as cancer therapeutics in most clinical trials and have shown an inexplicable short serum half-life, despite the lack of a HAMA response. The presence of anti-Gal antibodies in human serum ensures a quick removal of the xenogeneic mouse mAbs, containing Gal α 1-3Gal residues, which results in a lack of antibody-mediated effect on neoplastic target cells. Furthermore, even if the mouse mAbs can be detected in human serum after administration, the fact that they are present as immune complexes probably prevents them from recruiting normal effector functions, such as ADCC and complement-dependent cytotoxicity.

How do we then overcome this immunological barrier to successful antibody-dependent immunother-

Table 1. The Gal α 1-3Gal content of different mouse and human mAbs

Antibody	Antibody class	Antigen	Serum half-life (hours)	Relative anti-Gal reactivity ^a	Ref.
Mouse mAb					
17-1A	IgG2a/ κ	Glycoprotein	19	26.0	12
19-9	IgG1	Sialoyl-Lea	27	6.0	12
Adam	IgG1/ κ	EMBP	–	5.7	Björk, P., Donn, V. <i>et al.</i> unpublished
Anti-P	IgG2a/ κ	Progesterone	–	5.0	–
HMFG-1	IgG1	PEM (PDTR)	42	4.9	13
14.G2a	IgG2a	GD ₂	43	4.7	14
4H10	IgM	Glycoprotein	–	4.6	–
MB.36	IgG3	GD ₃	–	4.5	14
T10B9	IgM/ κ	T-cell antigen	–	4.2	–
HMFG-2	IgG1	PEM (DTR)	38	2.2	13
9.2.27	IgG2a	Proteoglycan	–	2.2	15
B72.3	IgG1	TAG.72/TnS	43	2.0	16
Human mAb					
MOE23 ^b	IgM/ κ	Digoxin	–	1.0	–
MOE26 ^b	IgM/ κ	Digoxin	–	1.0	–
MO96 ^c	IgM/ κ	V3 region/gp120	–	1.0	17
MO101 ^c	IgM/ κ	V3 region/gp120	–	1.0	17
LH1:92 ^c	IgM/ λ	Digoxin	–	1.0	18
LH1:114 ^c	IgM/ κ	Digoxin	–	1.0	18
MO6 ^c	IgM/ κ	Digoxin	–	3.1	18

The mouse monoclonal antibodies that were tested for endogenous glycosylation were coated onto microtiter wells and incubated overnight at room temperature. After washing the plates, human anti-Gal α 1-3Gal antibodies (isolated from human serum (Refs 7–10)) were added. The enzyme conjugate was horseradish peroxidase coupled to goat anti-human IgG, and o-phenylenediamine was used as chromogen.

The human monoclonal antibodies specific for digoxin or the V3-region were tested for endogenous glycosylation by using microtiter wells coated with a digoxin-BSA conjugate or goat anti-human IgM, respectively. The human monoclonal antibodies were then added to the washed wells. After a second washing step, biotinylated human anti-Gal α 1-3Gal antibodies were added to wells containing the anti-digoxin antibodies and unlabelled anti-Gal α 1-3Gal antibodies to wells containing anti-V3-region antibodies. The enzyme conjugate was horseradish peroxidase coupled to streptavidin or goat anti-human IgG, and o-phenylenediamine was used as chromogen.

^aThe anti-Gal reactivity index was calculated using:

$$\text{Anti-Gal reactivity} = \frac{\text{O.D. (mAb)} - \text{O.D. (background)}}{\text{O.D. (no mAb)} - \text{O.D. (background)}}$$

OD: optical density.

^b This human antibody was derived from a subclone of the EBV-transformed lymphoblastoid cell line used to establish the MO6 hybridoma¹⁸.

^c This human antibody was derived from a human-mouse-human hybridoma cell line.

Mouse monoclonal antibodies anti-P, 4H10 and T10B9 were kindly provided by Drs Cristina Glad and Bo Jansson at BioInvent International AB, Lund, Sweden.

apy? One possible solution would be to clone the mouse genes coding for the heavy and light chains of the mAb and express these genes in a host cell that does not have an active α 1,3-galactosyltransferase¹¹. The lack of this enzyme activity prevents the addition of Gal α 1-3Gal residues to all proteins pro-

duced. The above table also points to the importance of characterizing the glycosylation pattern of the expression host of choice, before using that host to express recombinant therapeutic antibodies. Finally, the data also lend support to the superiority of human monoclonal antibodies as immuno-

therapeutic agents, since human molecules are devoid of any Gal α 1-3Gal residues.

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Carl A.K. Borrebaeck, Ann-Christin Malmborg and Mats Ohlin are at the Dept of Immunotechnology, Lund University, Lund, Sweden.

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Erratum

In the August issue of *Immunology Today*, the authors affiliations were omitted from the article *The maturation of the immune response* on pp. 400-404.

We would like to point out that Claudia Berek and Mike Ziegner are at the Deutsches RheumaForschungsZentrum, Nordufer 20, D-13353 Berlin, Germany.

We apologize for any misunderstanding this has caused.

The Primary Immunodeficiency Association Research Fund 1993

The Primary Immunodeficiency Association (PiA) is the patient support organization and registered charity for those affected by primary immune deficiencies.

In 1993, the PiA will be funding research for the second year, when a total of £40,000 has been allocated for projects in clinical immunology.

Further details and application forms can be obtained from the general secretary of the PiA, Mr Robin Fanshawe, at the following address:

The Primary Immunodeficiency Association
PO Box 1490
Halstead
Essex, UK CO9 2SW
Tel. & Fax 0787 478299