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## DISCUSSIONS

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# Do YB2/0 Cells Produce Alien Sugars?

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**Abstract** Olovnikova et al. ( Impact on N-glycosylation profile of monoclonal anti-D antibodies as a way to control their immunoregulatory and cytotoxic properties (2012) *Biochemistry (Moscow)*, **77**, 925-933) mentioned the presence of alien sugars on monoclonal antibodies (mAbs) produced by YB2/0 cell line. We summarize in this paper our previous findings on the glycosylation profile of two anti-D mAbs produced in this cell line (LFB-R297 and LFB-R593, so-called Roledumab). Our results show the absence of any immunogenic glycotopes, and furthermore neither immunogenicity nor other serious adverse reactions were observed during clinical trials.

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**Key words:** YB2/0, monoclonal antibodies, anti-D, glycosylation, immunogenicity

We would like to discuss here a hypothesis reported by Olovnikova et al. in their original article published in *Biochemistry (Moscow)* that deals with the structure/function relationship of anti-RhD monoclonal antibodies ( Impact on N-glycosylation profile of monoclonal anti-D antibodies as a way to control their immunoregulatory and cytotoxic properties ) [1].

This interesting article focuses on the immunoregulatory and cytotoxic properties of anti-RhD antibodies with respect to their N-glycosylation profiles. The work concludes that an optimized glycosylation profile is needed to promote RhD-positive red blood cell clearance via interaction of anti-RhD antibodies with FcγRIIIa receptors. The use of YB2/0 cells as a way to produce efficient monoclonal anti-RhD antibodies is addressed.

During the last two decades, LFB Biotechnologies Research and Development teams have been focusing their efforts on designing therapeutic monoclonal antibodies with increased affinity to FcγRIII (CD16) receptors involved in antibody-dependent cell cytotoxicity (ADCC) and phagocytosis mechanisms. In this context, LFB Biotechnologies acquired specific expertise and knowledge in this field that allowed developing monoclonal antibodies characterized by a specific N-glycosylation pattern (low fucose) conferring increased interaction with FcγRIIIa (CD16) receptor expressed on NK cells and macrophages.

This property is notably obtained from the rat myeloma YB2/0 cell expression system and particularly due to its lower expression of *fut8* gene compared to other commonly used cell lines [2, 3] under appropriate culture conditions and clone selection. For large-scale production, the goal was to screen and select a highly productive and stable clone from the transfected population and to retain the product quality specifications such as low fucose content and high cytotoxicity in the presence of effector cells [4-6].

Olovnikova et al. [1] suggested that rodent cells may synthesize alien glycan structures, but the term alien does not refer to specific structures. To date, some monoclonal antibodies (mAbs) produced in murine-derived cell lines like NS0 or SP2/0 have been reported to contain glycoproteins bearing a variable amount of immunogenic glycotopes such as N-glycolyl neuraminic acid (NeuGc; H-D antigen) [7] and Gal(a1,3)Gal [8]. However, the Gal(a1,3)Gal glycotope is a major source of safety concerns in terms of hypersensitivity/anaphylaxis as it binds to circulating anti-Gal(a1,3)Gal IgE/IgG found in many humans [9, 10] arising from many ways of sensitization against Gal(a1,3)Gal-containing red meat and/or microbe-derived glycoconjugates [11]. It has also been reported that immunization against the Gal(a1,3)Gal-containing product was not observed when this epitope is present on antibody Fc domains [12]. YB2/0 cell line, derived from spleen cells

of a rat strain, has not been reported to produce such antigenic epitope so far.

The hypothesis about possible side effects (stimulation of immune response instead of its suppression) regarding putative alien sugars structures has been suggested on the basis of absence of publications, till 2008 [13], reporting the results of clinical trials with a low fucose mAb produced by YB2/0. However, during the submission time course of Olovnikova's paper, our group published in May 2012 the pharmacokinetics and safety results of a phase one clinical trial performed with Roledumab (LFB-R593), a new anti-RhD monoclonal antibody produced by YB2/0 cell line. Within this trial neither immunogenicity nor other serious adverse reactions were observed [14].

Olovnikova et al. [1] also mentioned a problem of low fucose anti-RhD production using different cell lines such as rodent cell lines. We published in 2006 the selection of a specific YB2/0 clone able of producing low core-fucosylated anti-RhD antibodies with enhanced FcγRIIIA-mediated activity [15, 16]. A glycosylation profile of this antibody is provided by Sibøril et al. [15] and shows classical complex glycan structures of type G0, G0F, G1, G1F, G2, and G2F common to polyclonal IgG but in different proportion (i.e. low fucose content). These glycan structures fulfill the structural requirements described by Olovnikova et al. for obtaining an anti-RhD mAb with appropriate safety and efficacy profile.

In their paper, Olovnikova et al. [1] discussed two potential safety concerns with anti-RhD mAb: immunogenicity due to the structure of particular sugars that can potentially lead to the generation of anti-drug antibodies (ADA), i.e. antibodies directed against the monoclonal antibody given to the subjects (pregnant women), and stimulation of the immune response leading to the generation of antibodies directed against RhD epitopes on RBCs (i.e. allo-immunization).

Two anti-RhD mAbs were produced by our group in YB2/0 cell lines, LFB-R297 and LFB-R593 (Roledumab) and tested in clinical trials. Both mAbs led to efficient clearance of RhD-positive red blood cells (RBCs) in RhD-positive healthy volunteers (infusion of R297-sensitized autologous RBCs [11]) as well as in RhD-negative healthy volunteers receiving 15 ml RhD-positive RBC challenge followed 24 h later by an administration of Roledumab [17]. RhD-positive RBC clearance rate was similar to that of the reference polyclonal anti-RhD with LFB-R297 [13] and greater with LFB-R593 (Roledumab) at a similar 300 g dose [17].

Since the report of Bøliard et al. [13], two new clinical trials have been conducted with Roledumab [14, 17]. In total, 79 subjects received Roledumab, from which ten were injected twice after a 6-month washout period. No allo-immunization nor anti-drug antibody reaction were

measured nor detected during the 6-month follow-up period whatever the route of administration (IV and IM) and the dose (from 30 to 3000 g/volunteer), indicating no activation of the immune system.

Roledumab is a promising human recombinant monoclonal anti-RhD antibody produced by a specific YB2/0 clone and using optimized upstream process conditions to provide high productivity and enhanced FcγRIIIA-mediated activity. Contrary to the hypothesis made by Olovnikova et al. in their paper [1], the glycosylation profile of anti-RhD mAbs, produced from YB2/0 cell line under well controlled and reproducible culture conditions, has not induced so far any immunogenic reactions and has not raised any safety concerns. Roledumab pharmacokinetics and safety profile will be soon evaluated in a pregnant women phase II clinical trial.

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