
DISCUSSIONS

Is an Expression System for Producing Therapeutic Antibodies with Immunosuppressive Properties Found at Last? Comment to Letter by Dr. Quagliaroli

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Abstract The prophylaxis of the hemolytic disease of the newborn a mandatory procedure in obstetrics requires significant amounts of plasma-derived polyclonal anti-D immunoglobulin. Despite numerous attempts, the proper technology for mass production of effective monoclonal anti-D is still not available. IFB Biotechnologies is currently performing clinical trials with recombinant anti-D antibody that has low fucose content and is expressed in the cells of rat myeloma YB2/0. It was shown that this drug is well tolerated, accelerates fast clearance of D+ red blood cells, and can inhibit anti-D immune response in Rhesus-negative volunteers.

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It was extremely interesting to read the letter by Dr. Quagliaroli (this issue) because it contains information that has been eagerly awaited by specialists working on the development of monoclonal preparations. The letter reports a real success in the creation of clinically active monoclonal antibodies (mAbs) against D antigen of the Rhesus (Rh) system necessary for preventing hemolytic disease of newborns. During the year after the publication of our paper in August 2012 [1], which motivated Dr. Quagliaroli to send the Letter to the Editor, the developers of a new preparation named Roledumab has obtained extremely important and promising results.

Polyclonal anti-Rh immunoglobulin prepared from sera of immune donors has long been used in clinical practice for prophylaxis of hemolytic disease of newborns caused by maternal anti-D antibodies. Polyclonal anti-D antibodies rapidly eliminate red blood cells of a positive fetus from the bloodstream of an Rh-negative woman that have entered her body during pregnancy or delivery, and they prevent the generation of her own anti-D antibodies. The story of creation of a monoclonal anti-D that can be used in clinical practice instead of the polyclonal preparation is rich with disappointments and failures. In studies on volunteers, human anti-D mAbs produced by CHO cells or human mouse heterohybridomas were either less effective than the polyclonal preparation or even caused an opposite effect [2]. Thus, in our experiments some

mAbs or their combinations promoted rapid clearance of D+ red blood cells from the bloodstream of Rh-negative donors, but these mAbs increased the frequency of immunization instead of preventing anti-D immune response [3]. Most likely these unsuccessful results were caused by an unsuitable profile of IgG glycosylation, because in cells from rodents, including the above-mentioned cell lines, glycans can be synthesized with structure different from that of natural glycans of human IgG. It seems that human mAbs produced in the cells of mouse myeloma and CHO are poorly recognized by the FcγRIIIA receptor, but they can be recognized by some other receptors (e.g. by innate immunity receptors), which leads to a proinflammatory response and immunization.

At the IFB Biotechnologies Company represented by Dr. Quagliaroli, recombinant low-fucosylated anti-D antibodies prepared via transfection of rat cell line YB2/0 are studied [4]. During recent years, cell lines deficient in the fucosyl transferase gene FUT8 or with an artificially suppressed activity of this gene are considered as an ideal cellular platform for creating cytotoxic preparations [5]. The absence of fucose on molecules of IgG class antibodies imparts to the Fc fragment high affinity for the FcγRIIIA receptor and the ability to destroy target cells due to attraction of FcγRIIIA-carrying effector immune cells (the ADCC reaction). Thus, low-fucosyl-

ated anti-CD20 mAbs destroy the target cells in ADCC much more effectively than the same mAbs produced by the CHO cells [6]. Expressing in YB2/0 cells anti-D mAbs, which initially did not react with the FcγRIIIA receptor, we prepared anti-D mAbs with high ADCC activity [7]. However, as differentiated from antitumor preparations, anti-D mAbs have to display more than a cytotoxic effect. The destruction of allogenic blood cells under the influence of anti-D mAbs has to be accompanied by development of tolerance to the D antigen, i.e. by inhibition of the anti-Rh immune response, as occurs under the influence of polyclonal anti-D antibodies. We still do not know the exact mechanism of development of this tolerance, and therefore we aimed at maximal imitation of the properties of the polyclonal preparation. In the paper of Sibøril [8] mentioned by Dr. Quagliarioli, a glycosylation profile is presented of recombinant mAbs produced in the YB2/0 cells, and this profile indicates that 100% of glycans of such mAbs correspond in the structure to human analogs but defucosylated. However, some literature data suggest that human mAbs prepared from YB2/0, upon injection into subjects under testing, can induce adverse effects (febrile reactions), as well as stimulate clearance of red blood cells without recognizing FcγRIIIA [9]. In this connection, we have a reasonable fear that even active mAbs can carry alien oligosaccharide structures because they are synthesized by rat cells. The letter of Dr. Quagliarioli is relevant just to this phrase of our paper, and it reports that during the last year LFB Biotechnologies Company has obtained convincing data that a preparation Roledumab created on the anti-D mAbs produced by the YB2/0 is not only safe [10], but can also accelerate the clearance of D+ red blood cells and prevent the alloimmunization of Rh-negative volunteers injected with 15 ml of D+ red blood cells and then with different doses of Roledumab. Results of clinical trials performed with 79 Rh-negative volunteers during six months have shown the efficiency of Roledumab. And this is a great success, showing the luck and skill of the creators of the preparation. During the whole history of clinical trials of different anti-D mAbs, this is the second successful monoclonal/recombinant immunoglobulin. But the first preparation obtained from cells transformed with the Epstein Barr virus [11] did not become a commercial preparation, whereas Roledumab is created on the basis of a stable and well characterized line suitable for a large-scale production. I sincerely hope that clinical trials with pregnant women will also be successful.

The creation of a monoclonal preparation for prophylaxis of anti-D immune response will promote such projects as the creation of a similar preparation for preventing alloimmunization of pregnant women against the platelet HPA-1a antigen [12], which does not have a polyclonal analog. In this disease, the maternal anti-platelet antibodies induce destruction of the platelets of

the fetus and development of thrombocytopenia of newborns. The prophylactic injection of anti-HPA-1 mAbs capable of inhibiting an undesired immune response would be promising as a directed prophylaxis of this severe disease, similarly to prophylaxis of hemolytic disease of newborns with anti-D antibodies.

The prophylaxis of hemolytic disease of newborns with anti-Rh immunoglobulin is a sore subject for Russia. The Russian preparation is produced in miserable quantities, and therefore this necessary procedure is often not performed in maternity hospitals. This results in a virtual absence of difference between the modern statistics of Rh-conflicted pregnancies and frequency of hemolytic disease in newborns in Russia and the corresponding data before the era of prophylaxis in advanced countries and does not demonstrate a tendency for decrease. Possibly, the LFB Biotechnologies success in the elaboration of the biotechnological anti-D preparation will stimulate Russian pharmaceutical companies to pay attention to this problem.

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