Production of scFvs as anti-ABO antibody antagonist for prevention of hemolytic transfusion reactions resulting from ABO blood group incompatibility between donor and recipient

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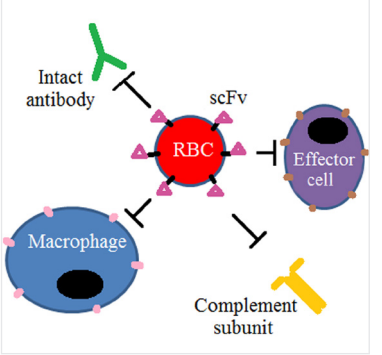

Illustration provided by Author

ABSTRACT ABO blood group incompatibility between donor and recipient is the main cause of hemolytic transfusion reactions (HTR). This is induced by the immunological route such as complement dependent cell lysis (CDC) and antibody dependent cell cytotoxicity (ADCC). The Fc region of the full-length antibody plays an important role in induction of these phenomena. Single chain fragment variable (scFv) antibody contains the complete antigen-binding site of an antibody molecule while it lacks the Fc domain and does not induce Fc-mediated functions including complement activation and antibody dependent cell cytotoxicity. Therefore these high affinity and specific antibody fragments are almost used as blocking agents. Our hypothesis is that scFv can be used as an antagonist of the whole anti-ABO antibody to inhibit red blood cell (RBCs) hemolysis. Therefore, the RBCs from all donors can be transfused to an individual patient.

BACKGROUND Hemolytic transfusion reactions (HTR) occur at an incidence of 1:76,000 transfusions[1]. ABO blood group incompatibility is the main cause of this reaction. Transfused red cells are destroyed by the immune system, because of incompatibility of antigens on donor red cells with anti-ABO antibodies in the recipient blood circulation[2]. This occurs due to administering blood to the wrong patient or testing errors[1].

HTRs involve intravascular and extravascular RBC destruction. Activation of the complement system that leads to intravascular hemolysis is mostly initiated by the binding of recipient patient antibodies to the blood group antigens on the surface of the transfused RBCs from the donor individual. This can lead to disseminated intravascular coagulation, renal failure, systemic shock and subsequently, death[3,4].

The classical pathway of complement activation is triggered by antigen-bound antibody molecules and is initiated by the binding of C1q to Fc domain of the antibody (Fig. 2A). All complement pathways converge at two levels: the C3 level, in which C3b is attached to the target cells, and at the C5 subunit level, in which C5b-C9 are polymerized on the cell surface and subsequently, a membrane attack complex (MAC) will be formed. After inserting the MAC into the cell membrane, the channel will remain open and consequently, water and ions are exchanged, and lethal colloid-osmotic swelling will occur which leads to target cell lysis[5].

RBCs that are coated by IgG, and not targeted by the complement system, are removed from circulation through destruction in the spleen. Such RBCs

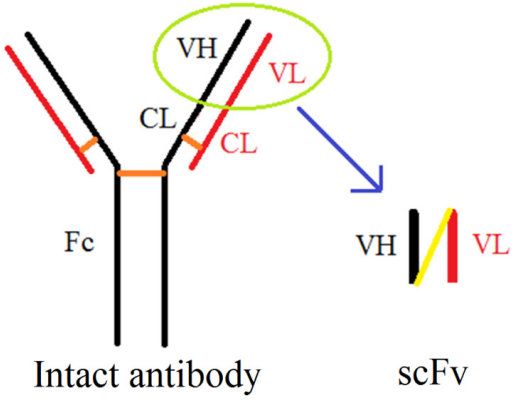

Figure 1 | Schematic representation of a full-length antibody (Left) and a scFv (Right).

can also be bound and phagocytosed by macrophages via opsonization and phagocytosis mechanisms. Opsonization involves the binding of an antibody to an antigen. After binding of opsonin to the cell membrane, phagocytes are attracted to the cell. While the antigen binding portion of the antibody binds to the antigen, the Fc domain of the antibody binds to an Fcγ receptor I (FcγRI) on the phagocyte, facilitating phagocytosis[6] (Fig. 2B).

Another mechanism of RBC destruction is performed by direct cell-cell contact with NK cells (Natural killer cells), neutrophils, eosinophils and macrophages (antibody dependent cellular cytotoxicity)[7](Fig. 2C). ADCC or the antibody-dependent cell-mediated cytotoxicity is an important and efficient mechanism of cellular-mediated defense of the immune system. In ADCC an effector cell of the immune system (NK cell, neutrophil or eosinophil) lyses a target cell, whose specific antibodies have been bound to the cell membrane antigens[8]. For instance, a NK cell mostly expresses FcγRIIIa receptors, which recognize and bind to the Fc domain of a cell surface bound antibody. Target cell lysis and destruction will occur by attached NK cells releasing specific proteins, such as perforin[9].

Single chain fragment variable (scFv) (Fig. 1) is the most common recombinant antibody variant, and contains the complete antigen-binding site of an antibody which lacks the Fc domain[10]. In the structure of these molecules there are two variable domains, variable heavy (VH) domain and variable light (VL) domain that are connected by a flexible polypeptide linker. They have shown improved pharmacokinetic properties better than whole antibodies[11,12]. The absence of Fc domain has additional advantages: they do not induce functions related to Fc domain such as antibody-dependent cell cytotoxicity or complement-dependent cytotoxicity[13].

The human origin, small size and high affinity properties of scFvs have made these antibodies ideal agents for immunotherapy purposes. Due to unique characteristics of scFv, we proposed our hypothesis using these antibodies as effective blockers regarding blood transfusion problems.

HYPOTHESIS We hypothesized that single chain fragment variables can be used as antagonist molecules against whole anti-ABO blood group antibodies. We assume that by blocking ABO and other blood group antigens using scFvs and preventing hemolysis by anti-ABO antibodies (Fig. 3), no cross-match would be needed, and one patient could receive blood cells from all donors.

The evidence to support the hypotheses are discussed in three parts. First, by specific data on adipose compensation, second by additional data on energetic substrates and tissue compensation, and finally possible mechanisms to explain the hypotheses are proposed.

EVALUATION OF HYPOTHESIS

PANNING PROCESS The procedure of panning is performed using a scFv human phage display library against the desired epitope as follows. In each round of panning, the proteins are coated on immunotube. The tube is washed several times with PBS and blocked with skimmed milk or BSA. Phages are added and incubated at room temperature. Following washing, bound phages are eluted with log-phase TG1 *E. coli.* After rescuing the new phages transformed *E. coli* with helper phage M13KO7, several further rounds of panning are performed against the antigen[14,15].

POLYMERASE CHAIN REACTION (PCR) After the last round of panning, PCR is performed to investigate the presence of an expected band corresponding to scFv gene insert.

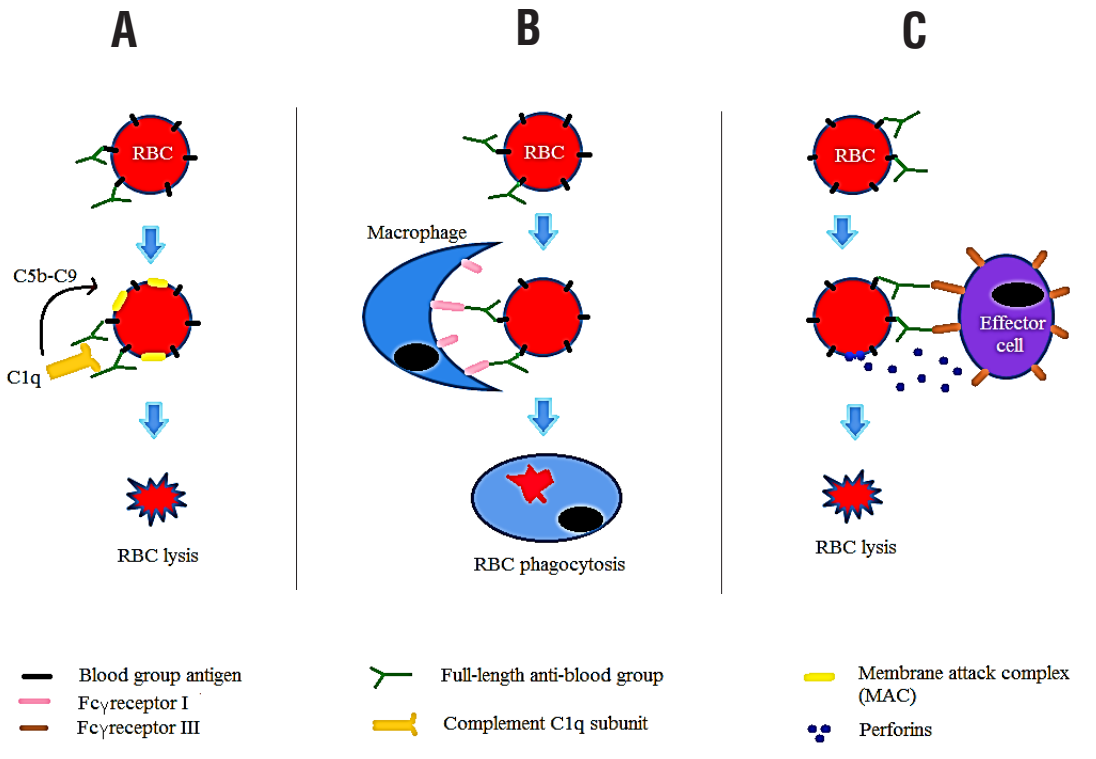

Fig. 2 | Schematic depicting immunogenic responses leading to RBC lysis. Anti-incompatible blood group antibody can bind to the RBC surfaces via blood group antigen and result in blood cell lysis due to A; CDC (Complement dependent cell lysis) B; phagocytosis and C; ADCC (antibody dependent cellular cytotoxicity) mechanisms.

DNA FINGERPRINTING Fingerprinting is carried out on almost 20 colonies after the last round of panning. Each PCR product is digested with MvaI restriction endonuclease and run on agarose gel. Fingerprinting is generally used to determine enriched and selected clones following panning.

PHAGE *ELISA* To evaluate the efficacy of the panning selection process and also determine the binding ability of a single clone binder phage derived from panning, the resultant phages are tested by phage ELISA. Binders were detected using a HRP conjugated anti-fd (Filamentous phage) antibody in combination with colorimetric substrate.

ScFv ANTAGONISTIC EFFECT ASSESSMENT The antagonistic or blocking effect of the selected anti-blood group antibody fragment (scFv) is assessed by preventing the RBCs agglutination and complement activation which occur by intact antibodies in the laboratory.

IN VIVO EVALUATION To investigate the homologous or xenogeneic RBC destruction mediated by immune system, different types of animal models have been used and evaluated, so far. There are many transgenic and knockout mice models to evaluate the mechanisms regarding HTRs[4].

DISCUSSION Anti-A and anti-B blood group antibodies are present in individuals lacking the corresponding antigen. The immune response from an incompatible blood transfusion is rapid and can lead to severe complications and death[16].

The Fc (Fragment crystallizable) domain is the C-terminal region of an intact antibody that interacts with Fc receptors on the surface of several immune cells such as macrophages and NK cells and also interacts with the several complement system members. The immune system can be activated using this unique property of antibodies[17]. Lack of Fc domain in antibody fragments such as scFv provides some advantages including that these antibodies do not induce

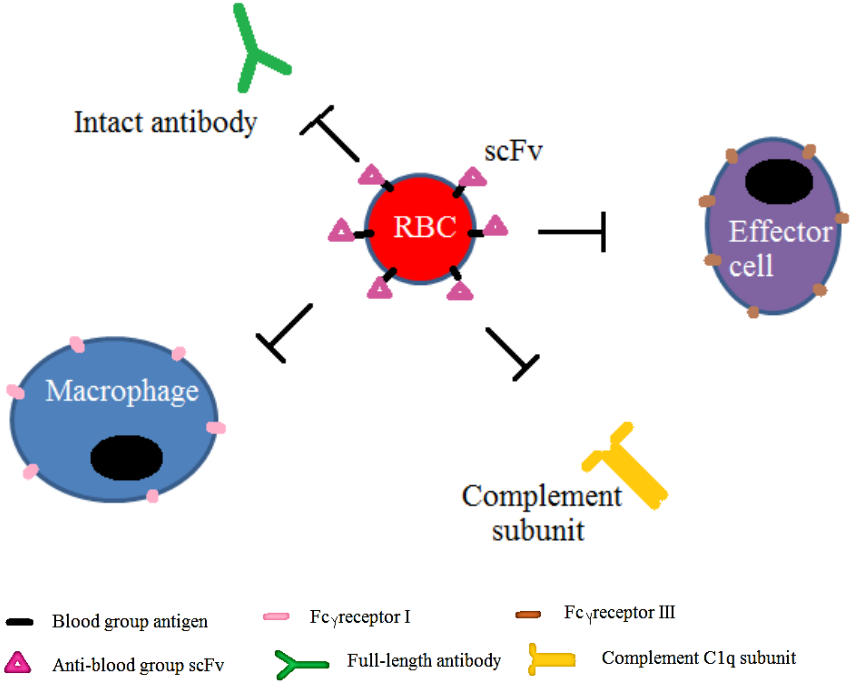

Fig. 3 | Anti-incompatible blood group scFv as an antagonist for full-length antibody can bind to the RBC surfaces via blood group antigen and inhibits the binding of full-length antibodies. Afterward, it blocks the ADCC, phagocytosis and CDC mechanisms.

functions related to the Fc region such as ADCC and CDC[13]. To isolate antibody fragments against the cell surface markers such as blood group antigens, the antibody phage display method has been used, successfully[18,19]. So far, several scFv antibodies have been isolated and selected against the blood group antigens[18, 20-22]. Furthermore, several studies have demonstrated the antagonistic or blocking properties of scFvs against the different molecules[23-26].

Here we present the hypothesis that high affinity scFvs can be used to block blood group antigens. These high affinity scFvs are proposed to be administered before blood transfusions thereby blocking the binding of alloantibodies present in the blood circulation of patients. Therefore, due to lack of the Fc domain in scFvs the complement dependent hemolysis and ADCC phenomena will not occur. In addition, scFvs have one antigen binding domain, so no RBC agglutination will occur due to binding of antibody fragments to RBCs, which is a reason for accumulation and agglutination of RBCs when whole antibodies with two antigen binding units are used. If the specific anti-blood group scFvs are to be applied, the red blood cells of a donor would be administered to a recipient with other blood groups.

However, there are several problems with this idea: 1) Stability of scFv molecules in blood bags may be challenging. In this case, some residues which have important roles in the stability of scFv proteins can be substituted using protein engineering[27-30]. Moreover, anti-ABO scFv antibodies can be injected into the blood bags just a few moments before administration of the blood cells to the recipients. 2) The cost of this product might be a problem but because of easy and abundant production of scFvs in bacterial hosts, the cost can be decreased[31]. 3) The minor blood groups in different individuals should be considered. 4) Although the use of animal models to study the immune response to incompatible RBC transfusions is not new, each of these has problems[4]. **END**

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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