Human antibodies for the generation of new red blood cell antigen typing reagents.

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Solve above mentioned problems by cloning new and existing IgG-based monoclonals for repurpose them as recombinant directly agglutinating IgM typing reagents.

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Other condition
Study type	Observational invasive

Summary

ID

NL-OMON50548

Source
ToetsingOnline

Brief title
Development of recombinant antibodies

Condition

- Other condition

Synonym
alloimmunization, Blood transfusion

Health condition

Patienten met antistoffen gericht tegen rode bloedcel antigenen

Research involving
Human

Sponsors and support

Primary sponsor: Sanquin Bloedbank
Source(s) of monetary or material Support: Sanquin

Intervention

Keyword: antibodies, recombinant

Outcome measures

Primary outcome

Monoclonal antibodies generated with recombinant-DNA technology from antibody

producing B-cells from patients with RBC antibodies.

Secondary outcome

Not applicable

Study description

Background summary

Monoclonal antibodies are used for red blood cell (RBC) antigen typing of patients and donors, routinely by using agglutination-based assays. Correct typing is of utmost importance for safe blood transfusions. For rare blood group antigens, only limited antisera are available, and some of them are not reliable. In other cases, available typing reagents are of the IgG type while IgG antibodies are not functionally adequate. IgG antibodies do not lead to good agglutination and to compensate for that a Coombs reagent needs to be applied (anti-IgG) to enhance the agglutination. These problems can be circumvented when the testing reagent is an *anti-blood group* antibody of the IgM isotype, which can be used directly to induce agglutination. Most importantly, current supplies of available antibodies against blood groups will become unavailable in 3-5 years. There is therefore a compelling need to generate a new and unlimited supply of monoclonal antibodies that can be used for RBC antigen typing.

Study objective

Solve above mentioned problems by cloning new and existing IgG-based monoclonals for repurpose them as recombinant directly agglutinating IgM typing

reagents.

Study design

When at Sanquin Immunohematology Diagnostics an antibody of interest is detected in a patient, the treating physician will be contacted to ask the patient if the researcher is allowed to ask the patient for participation. After approval, the Sanquin Researcher will contact the patient for participation in the research. After informed consent (see F1) is obtained a one-time blood sample of 36 ml will be taken. Considering the patient's preference, sampling will be done at a Sanquin location or by the general practitioner. In case of the latter a package with all necessities for blood sampling will be sent to the patient.

The antibodies of interest (not exclusievly) are anti-Fya, -Fyb, -Kpa, -Kpb, -Lua, -Lub, -Xga, -Wra, -Cob and -s.

From the patients* mononuclear cells, all CD19+ B cells are purified. To enrich for antibody-specific B cells, antigen specific erythrocyte ghosts are added to CD19+ B cells and all antibody-specific B cell-ghost cell complexes are sorted as single cells in 96-well flat-bottom plates. No viable cells of the patient will be stored. The B cells are cultured for 7 days, after which supernatants are harvested and tested for agglutination with a panel of antigen positive and negative RBC. From the progeny of the cells that produced the antibody, RNA is extracted and reverse-transcribed. During this RNA extraction procedure, the cultured patient cells are lysed, so no viable patient cells will be left. The cDNA is amplified and PCR products are sequenced and cloned into vectors containing the variable kappa and each of the four human IgM or IgG subclasses constant regions. HEK293 (Human Embryonic Kidney) cells are transfected with DNA, cultured for 5 days and IgG and IgM antibodies are purified from the supernatant. These recombinant vectors can be multiplied to generate an unlimited supply of the antibodies. The original plasma of the patient containing the polyclonal alloantibodies will be stored, to compare reactivity with the recombinant antibodies.

As a proof of concept, we recently successfully applied these techniques to produce a unique anti-Vel-specific IgM class antibody, that was able to distinguish between Vel-negative and very weak Vel antigen expressing RBC by direct agglutination
(reference: van der Rijst MVE, Lissenberg-Thunnissen SN, Ligthart PC, Visser R, Jongerius JM, Voorn L, Veldhuisen B, Vidarsson G, van den Akker E, van der Schoot CE. Development of a recombinant anti-Vel immunoglobulin M to identify Vel-negative donors. Transfusion. 2019;59:1359-1366).

Study burden and risks

Patients will undergo a one time venipuncture for the collection of 36 ml blood.
A venipuncture is regarded an intervention with minimal risks

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Patients (at least 18 years of age) who formed antibodies against RBC antigens.
The specificity of these antibodies have been determined by Sanquin Immunohaematology Diagnostics.

Exclusion criteria

Patients below the age of 18, incapacitated patients and patients who cannot understand the information

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	30-06-2020
Enrollment:	30
Type:	Actual

Ethics review

Approved WMO

Date:	05-02-2020
Application type:	First submission
Review commission:	METC Amsterdam UMC

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL64327.018.19