Effects on the anti-ABO titers of military blood donors from a predeployment vaccination program

Olle Berséus, MD, PhD, *Örebro, Sweden*

BACKGROUND:	The use of blood group O as "universal blood" for emergency whole blood transfusions carries the risk for a hemolytic transfusion reaction mediated by incompatible A/B antibodies. This risk can be minimized by assuring that the donor has a low titer of anti-A and anti-B. The level of these naturally occurring antibodies has been shown to be increased by vaccination with most biologically derived vaccines. This boostering effect has been investigated for the new generation of vaccines.
METHODS:	The 120 crew members of a Swedish naval ship deployed for 7 months to the Indian Ocean were tested for anti-A and anti-B before their predeployment vaccination program and after returning to Sweden. The vaccination program contained vaccines against cholera, diphtheria, hepatitis A and B, influenza, measles, meningitis, mumps, pertussis, polio, rubella, TBE virus, tetanus, typhus and yellow fever. Paired antibody titrations were performed for both IgM and IgG using microtube gelcards (Diamed GMBH).
RESULTS:	No crew member, including the six belonging to the "high titer" group, showed a sign of a booster effect by any of the used vaccines.
CONCLUSION:	The earlier reported boostering effects mediated by different vaccines cannot be replicated with the new vaccines of today. This is probably a result of the new manufacturing techniques resulting in much purer vaccines. (*J Trauma Acute Care Surg.* 2017;82: S91–S95. Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.)
LEVEL OF EVIDENCE:	Therapeutic/care management study, level II.
KEY WORDS:	Blood group O donors; universal blood donor; isoagglutinins; anti-A anti-B titers; vaccination.

Both in the military and civil medical service the use of blood group O as "universal blood" for emergency whole blood transfusions is increasing.[1,2] As all group O whole blood units contain the isoantibodies anti-A and anti-B this transfusion may induce a clinically significant acute hemolytic transfusion reaction. To minimize the risk all blood group O "universal donors" must have a low titer of anti-A and anti-B.[3] Even if the regulatory agencies in most countries have specified the cutoff titer values for group O donors accepted as "low titer" and thus as a universal donor, there is no internationally accepted optimal technique for the titration or levels of the cutoff values.[4–8] However, titrations using the microtube gel card technique manually or automated is widely used.

An early recognized problem has been the boostering effect on the anti-A and anti-B production of vaccinations against infectious diseases. This has been described for typhoid-paratyphoid vaccine[9] and tetanus vaccine,[9,10] influenza vaccine,[11,12] pneumococcal vaccine,[13–15] and plague vaccine.[16,17]

Because the ABO blood group substances are shared with many common bacteria and viruses,[18,19] a biologically derived vaccine may contain contaminants showing an ABO antigen capacity. In addition, substances with A and B substance-like antigen capacity may be entered via the culture media or other additives during the manufacturing procedure. An example is A-like substance in influenza vaccine which is reported to originate from the embryonated chicken eggs and the viruses grown in them.[20] In the case of the pneumococcal vaccine, the A-like substance has been identified as one of the virus antigens which could be eliminated by purification without loss of the influenza protecting power of the vaccine.[21–23] The clinical significance of the boostering effect has been documented in a number of publications covering several of the regularly used earlier vaccines.[24–26] In contrast, most of the new vaccines used today are biosynthetically produced and/or much better purified and thus much less contaminated with blood group antigenic substances. In view of the increasing use of fresh group O blood for transfusion, it was of interest to evaluate the eventual booster effect on A/B-antibodies after vaccinations using vaccines of the new generation.

MATERIALS AND METHODS

The Swedish naval vessel HMS Carlskrona that was deployed for 7 months to the Indian Ocean during 2013 was chosen for the study. After individual acceptance to participate, the whole regular crew of 98 men (age, 20–62 years; median, 34 years) and 22 females (age, 21–56 years; median, 25 years) joined the study. Crew members belonging to the special forces were excluded from the study. Before the vaccinations (the vaccines used are listed in Fig. 1), a blood sample from each crew member was collected, centrifuged, and serum was separated and directly frozen at −22°C. At the health check after the return of the ship to the home port, a second blood sample

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From the Department for Transfusion Medicine (O.B.), Örebro University Hospital, Örebro, Sweden.
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Address for reprints: Olle Berséus, MD, PhD, Department for Transfusion Medicine, Örebro University Hospital, Örtenlundsvägen 7, 02 30 Örebro, Sweden; email: berseus@telia.com.

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Crucell Sweden AB, *Stockholm, Sweden.* *infocrucellse@its.jnj.com*
Dukoral
Cholerae toxin B-subunit (rCTB)
Vibrio cholerae 01 Inaba, El Tor biotype (formalin inactivated)
Vibrio cholerae 01 Inaba, classic biotype (heat inactivated)
Vibrio cholerae 01 Ogawa, classic biotype (formalin inactivated)
Vibrio cholerae 01 Ogawa, classic biotype (heat inactivated)

Vivotif
Salmonella typhi, live attenuated (strain Ty21a)

GlaxoSmithKline AB, *Solna Sweden,* *infoprodukt@gsk.com*
Twinrix
Hepatitis A-virus, inactivated
Hepatitis B-surface antigen

Boostrix Polio
Diphtheria toxoid
Hemagglutinin (FHA), purified, philamentous
Pertactin
Pertussis toxoid
Poliovirus type 1, inactivated strain Mahoney)
Poliovirus type 2, inactivated (strain MEF 1)
Poliovirus type 3, inactivated (strain Saukett
Tetanus toxoid

Engerix-B
Hepatitis-B surface antigen

Havrix
Hepatitis A-virus, inactivated

Sanofi Pasteur MSD AB, *Solna, Sweden,* *infose@spmsd.com*
Stamaril
Yellow fever virus, live (strain 17d)

M-M-R VaxPro
Measles virus, Enders' Edmonston strain (live, attenuated)
Mumps virus, Jeryl Lynn™ [Level B] strain (live, attenuated)
Rubella virus, Wistar RA 27/3 strain (live, attenuated)

Vaxigrip
Influenza virus A/California/7/2009 (H1N1)pdm09-derived stam
(NYMC X-179A), split virus, inactivated (2)
Influenza virus A/Victoria/361/2011 (H3N2)-like strain
(NYMC X-181, derived from A/Texas/50/2012), split virus, inactivated (4)
Influenza virus B/Massachusetts/2/2012, split virus, inactivated (2)

Imovax Polio
Poliovirus type 1, inactivated (strain Mahoney)
Poliovirus type 2, inactivated (strain MEF 1)
Poliovirus type 3, inactivated (strain Saukett)

Typhim Vi
Salmonella typhi,Vi polysaccharide

Baxter Medical AB, *Kista, Sweden* *info@baxter.se*
FSME-IMMUN
TBE-virus antigen

Novartis Vaccines and Diagnostics S.r.I, *Siena, Italy* *service.uk@novartis.com*
Menveo
Meningococcus group A-oligosaccharide
Meningococcus group C-oligosaccharide
Meningococcus group W-135-oligosaccharide
Meningococcus group Y-oligosaccharide

Figure 1. Vaccines used in the vaccination program for the 2013 deployment of HMS Carlskrona.

was collected (8–9 months after the first sample) and serum prepared as described above.

After thawing all the samples, except those from blood group AB, were titrated in pairs for their respective anti-A and anti-B of both IgM and IgG type.

All titrations were performed using microtube gel cards (Diamed NaCl/enzyme and cold aggl for IgM and Diamed Coombs for IgG. Diamed GMBH, Cressier, Switzerland). For IgM titrations, the serum sample was diluted 1:100 with isotonic saline. For the titration of IgG the serum samples were incubated with an equal volume of 0.01 M dithiotreithol (Sigma-Aldrich Sweden AB, Stockholm, Sweden) for 45 minutes at 37°C and thereafter diluted 1:400 with isotonic saline. The 1% suspensions of A- and B-test cells where freshly prepared in Cellstab (Diamed GMBH) and checked according to the laboratory routine of the blood center. All series of titration contained a positive control in form of a sample from a locally produced and calibrated frozen serum pool and were read manually. For individuals showing samples with agglutination at a dilution of 1:100 for IgM or 1:400 for IgG, serial dilutions were prepared from these samples to determine the highest titer with agglutination and analysed as described above. If the corresponding A antibody or B antibody had shown a titer lower than the above titers, the dilution steps only included 1:50, 1:100, 1:200 for IgM and 1:200, 1:400, 1:800 for IgG.

In Sweden, a negative agglutination at a dilution of 1:100 for IgM and 1:400 for IgG is used as cutoff for the selection of "low titer" group O donors to be used as donors for universal group O blood.

RESULTS

The distribution of blood groups in the study group was group A, 63; group B, 11; group AB, 3; and group O, 43. Serum samples from all crew members, except those with group AB, were titrated for anti-A and or anti-B as described above. Only six (5%) individuals of the 117 titrated individuals showed a positive agglutination at the respective IgM (1:100) or IgG (1:400) dilutions representing the "high titer" group. All six were positive with a high titer both before and after the vaccinations and were further investigated with a complete serial titration, and the resulting titers are shown in Table 1. None of the investigated crew members showed an increase in the titer of anti-A or anti-B in the postvaccination sample.

DISCUSSION

In the earlier publications discussing the origin of the naturally occurring isoantibodies anti-A and anti-B, it was clearly shown that biological extracts from bacteria, viruses and also many of the clinically used vaccines contained substances with an A/B-antigenic capacity.[9–19] The immunogenic stimulation of the production of anti-A and anti-B could be shown to have clinically significant implications and even induce a hemolytic transfusion reaction. A study by Gupte and Bhatia[26] showed that antitetanus vaccine containing purified tetanus toxoid caused an immunological stimulation of anti-A and anti-B of Ig-G type when given to pregnant blood group O mothers leading to an increased frequency of hemolytic disease of the newborn in the corresponding children. In a blood group O donor, this boostering effect of the vaccination has resulted in a hemolytic transfusion reaction in the recipient of the donated blood product caused by the high titer of incompatible antibodies in the donor's plasma.[24,25]

As has been clearly demonstrated for the pneumococcal vaccines, the A- and B-like substances in the vaccines are not essential to the protective capacity of the respective vaccine but could be seen as a contaminant or impurity.[21,22] The possibility of not intended immunostimulations has been recognized by the manufacturers and resulted in new highly purified or biosynthetic vaccines where this type of contaminants are very much reduced or even eliminated. An example is vaccines produced using the new technique with a variant COX virus with the genes for the respective immunogen inserted in the virus genome as vehicle for the immunization.[27] Our study was set up to investigate if these new manufacturing techniques also reduced the boostering effects of A- and B-like substances after the vaccination in a group of blood donors. The predeployment vaccination program included a regular vaccination with 13 different vaccines which are specified in Figure 1. None of the examined

TABLE 1. Titrations of Anti-A and Anti-B in the Individual Crew Members Showing a Titer in the High Titer Area

	Serum Dilution Showing a Positive Agglutination			
	Sample Prevaccination		Sample Postvaccination	
Crew Member (Blood Group, Age)	IgM	IgG	IgM	IgG
1 (A, 23-y female)	anti-B 1:100	anti-B <1:400	anti-B 1:100	anti-B <1:400
2 (O, 34-y male)	anti-A 1;400	anti-A 1:800	anti-A 1:400	anti-A 1:400
	anti-B 1:200	anti-B 1:400	anti-B 1;200	anti-B 1:400
3 (O, 23-y male)	anti-A 1:100	anti-A <1:400	anti-A 1:100	anti-A <1:400
	anti-B <1:100	anti-B <1:400	anti-B <1:100	anti-B <1:400
4 (O, 62-y male)	anti-A <1:100	anti-A 1:400	anti-A <1:100	anti-A 1:400
	anti-B <1:100	anti-B <1:400	anti-B <1:100	anti-B <1:400
5 (O, 24-y male)	anti-A 1:200	anti-A <1:400	anti-A 1:200	anti-A <1:400
	anti-B 1:100	anti-B <1:400	anti-B 1:100	anti-B <1:400
6 (O, 23-y male)	anti-A 1:100	anti-A 1:400	anti-A <1:100	anti-A 1:400
	anti-B <1:100	anti-B <1:400	anti-B <1:100	anti-B <1:400

crew members showed an increase of the levels of anti-A or anti-B. One strength of this study is that the samples from each individual were titrated in pairs on the same microtube gel card and thus avoiding the rather great variation in the titration methodology depending on lack of a standardization of the group A and B test cells (unpublished results from the internal quality program of the laboratory). Our results are consistent with the reported findings that neither rabies vaccination[28] nor influenza vaccination[29] with vaccines of today show any effect on the formation of anti-A or -B. A limitation of the study is the absence of samples taken during the first month after the vaccination due to logistic problems during the deployment. Thus, a transient titer increase cannot be excluded. However, as the immunizing agent is presented to the immune system through an injection, the immunization process can be compared with the immunization by an ABO-incompatible pregnancy. At a new pregnancy after one with an immune caused jaundiced baby, the mother is routinely titered for the responsible antibody early in the pregnancy, and usually, the new titer is in the same level as after the previous pregnancy (personal observation). This could implicate that the injection of an ABO immunogen would result in a longstanding immune anti-A-/B antibodies, and thus a transient antibody peak seems less probable.

The found frequency of 5% "high titers" represents regular Swedish military personnel and corresponds to the earlier reported frequency in normal Swedish blood donors. In the Swedish Special Forces, preliminary data indicate a frequency of "high titers" of about 25%.[3] Due to the fact that the proposed background for the vaccination-induced booster effects on the ABO-antibody production are contaminants or impurities in the respective vaccine, there should not be a reason for any systematic difference in how the anti-A and/or anti-B titers are affected by a vaccination with regard to ethnicities or geography.

A dietary intake of probiotics has been described to induce a booster effect on the production of anti-B in a group O donor resulting in a hemolytic transfusion reaction in two recipients.[30] As in this case, the immunization agent is introduced via the gut the immune process may follow a different path and the time span for the corresponding antibodies should be investigated.

CONCLUSION

In this investigation, there was no sign of a booster effect on the level of ABO antibodies from the used vaccines. Compared with the earlier published reports showing clear boostering effects mediated by different vaccines, our findings are probably a result of using the much more pure vaccines of today. If our results can be corroborated in a larger study, it may be possible reduce the number of titrations for ABO antibodies of blood group O "universal donors" by eliminating the need for retitration after a vaccination program and thus simplify the handling of this donor pool in a walking blood bank.

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DISCLOSURE

The author declares no conflicts of interest.

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