AUTOIMMUNE COMPONENT IN INDIVIDUALS DURING IMMUNE RESPONSE TO INACTIVATED COMBINED VACCINE AGAINST Q FEVER

L. KRUTITSKAYA[1], N. TOKAREVICH[1], A. ZHEBRUN[1], N. KARTSEVA[1], I. TARASEVICH[2], V. YABLONSKAYA[2], S. FATALIEVA[2], O.P. MISNIKOV[3], A.Z. VASILENKO[3]

[1]Pasteur Institute of Epidemiology and Microbiology, St. Petersburg 197101; [2]Gamaleya Research Institute of Epidemiology and Microbiology, Moscow; [3]Institute of Military Medicine, St. Petersburg, Russia

Received September 18, 1995; revised July 22, 1996

Summary. – Serum samples from 20 individuals immunized with inactivated combined vaccine (ICV) against Q fever and 10 individuals that received placebo were investigated on days 14, 21, 28 and 60 after immunization by isotype specific enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies directed to human IgA, IgM and IgG, and their fragments ($F(ab')_2$, Fab, Fc). None of the subjects that received placebo exhibited significant increase of reactivity with any of the used antigens. By contrast, the sera of immunized individuals tended to show increased autoantibody activity with diverse antigens. Forty % of sera of immunized subjects exhibited anti-Fab activity, 20% of the sera recognized IgA, $F(ab')_2$- and Fc-fragments, and 15% of the sera recognized IgG and IgM. Although there was wide variation in antibody levels and in isotypic heterogeneity of autoantibodies induced by immunization, anti-Fab autoantibodies were represented mainly by IgG and IgA isotypes but not IgM isotype. A direct correlation between the anti-*Coxiella burnetii* (anti-*C.b.*) antibody level and the anti-Fab IgG activity, and between the anti-*C.b.* antibody level and the anti-Fab IgA activity was found. In the group of vaccinees reacting strongly to the vaccine against Q fever, this correlation significantly increased for both the anti-Fab IgG and the anti-Fab IgA activities. No correlation was found with the sera in the group of the subjects that received placebo.

Key words: Q fever vaccine; *Coxiella burnetii*; autoantibodies; IgA, IgM, IgG; fragments of immunoglobulins

Introduction

Although the phenomenon of the autoantibody production induced by experimental immunization and vaccination of individuals has been described, the origin and biological role of autoantibodies remain unknown. It has been shown that the vaccination of normal adults with tetanus toxoid increases the frequency of rhematoid factor (RF) precursor B lymphocytes (Welch *et al.*, 1983). Recent data indicated autoantibodies against insulin and thyreoglobulin in the children immunized with rubella vaccine (Bodansky *et al.*, 1989). We have previously shown that the immunization of individuals with influenza vaccine and staphylococcus anatoxin induced autoantibodies against some serum proteins – immunoglobulins A, M, and G, and their fragments (Zhebrun *et al.*, 1991).

In the present report we have examined the possibility of induction of autoimmune phenomenon during the immune response to an antigen of another nature – ICV against Q fever.

Abbreviations: BSA = bovine serum albumin; *C.b.* = *Coxiella burnetii;* CFT = complement fixation test; ELISA = enzyme-linked immunosorbent assay; ICV = inactivated combined vaccine; IIFT = indirect immunofluorescence test; PAGE = polyacryalamide electrophoresis; PBS = phosphate buffered saline; s.c. = subcutaneous(ly); SDS = sodium dodecyl sulphate

Materials and Methods

Immunization and serum samples. Serum samples from 20 healthy individuals immunized with vaccine against Q fever were investigated. The new vaccine preparation – ICV from phase I *C.b.* of Luga-1 strain, which underwent 3-4 chicken embryo yolk sac passages (Yablonskaya *et al.*, 1994), was used for the immunization. ICV contained two components: highly purified corpuscular antigen of *C.b.* (16 μg) inactivated by formalin, and soluble fraction of *C.b.* (10 AU) obtained by ultrasonic disintegration (Tarasevich *et al.*, 1995). According to our data, ICV showed weak reactogenicity and sufficient immunological efficacy in trials on humans, but the individual components of ICV exerted a weaker immunogenic activity than the complete ICV. The subjects were immunized subcutaneously (s.c.) once with optimum dose. Sera were collected prior and on days 14, 21, 28 and 60 after immunization. 10 control individuals were injected 0.5 ml of phosphate buffered saline (PBS).

CFT, IIFT and ELISA. To follow the dynamics of antibodies against *C.b.* in the tested sera, we used complement fixation test (CFT), indirect immunofluorescence test (IIFT) and enzyme-linked immunosorbent assay (ELISA). CFT and IIFT were carried out employing the standard techniques. As positive were scored those sera, which showed positive results at dilutions of 1:10 or greater in CFT and at dilutions of 1/8 or greater in IIFT. ELISA was performed as described previously (Gorbachev *et al.*, 1989) with the corpuscular antigen of phase I *C.b.* obtained according to the method of Kováčová *et al.* (1987). Titers of antibodies were calculated from A_{492} readings. The anti-*C.b.* antibody titer was defined as that maximum dilution of the serum whose A_{492} was higher than two-fold of A_{492} of the serum taken from the same subject prior immunization.

Detection of autoantibodies in the serum samples was performed by ELISA using highly purified human IgA, IgM and IgG, and their fragments as coating antigens.

IgG were isolated and purified from healthy human sera as described by Brock (1987). IgM were isolated from myeloma blood plasma using the method of Jehanly *et al.* (1981) in our own modification. IgA were isolated and purified from myeloma blood plasma by our own method (Zhebrun *et al.*, 1992). Fab- and Fc-fragments of human IgG were obtained by papain digestion of IgG using the method of Porter (1959), followed by chromatography on protein A-agarose and gel filtration on Sephadex G-100 (Pharmacia). $F(ab')_2$-fragments of human IgG were prepared by pepsin hydrolysis of IgG (Brock, 1987) followed by gel filtration on Sephadex G-100 and G-200. All these preparations were shown to be pure by immunoelectrophoresis and by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate (SDS).

ELISA autoantibody activity. Microtiter plates (Biohit, Finland) were coated with soluble antigens (IgA, IgM, IgG, $F(ab')_2$, Fab- or Fc-fragments) in 0.1 mol/l glycine-buffered saline pH 8.2 in concentrations determined previously. Protein concentration of the coating antigens was usually 5 – 10 mg/ml; however, a concentration of 100 ng/ml was shown to be adequate for detection of IgG antibodies against IgG and their fragments. After incubation of dilutions of sera in the antigen-coated wells, the bound autoantibodies were revealed with a 1:1000 dilution of a peroxidase-labelled rabbit anti-human IgG, IgM or IgA (Fc-specific) antibodies (Pasteur Institute, St. Petersburg). All dilutions were made in PBS pH 7.2 containing 0.1% bovine serum albumin (BSA), 0.5mol/l NaCl and 0.1% Tween 20. All incubations were carried out at 37°C for 1 hr. All steps described above included three washings in PBS containing 0.5 mol/l NaCl and 0.1% Tween 20. After the last washing, the peroxidase substrate (0.4 mg/ml o-phenylendiamine dissolved in 0.1 mol/l sodium acetate buffer pH 5.0 containing 0.02% H_2O_2) was added and the plates were incubated for 30 mins at room temperature. A_{492} was read using a multiscan automatic plate reader.

Data analysis. The mean A_{492} from duplicate assays was calculated. Values obtained with serum dilutions of 30 sero-negative healthy individuals were used for the determination of the cut-off value (2 SD above the mean). A serum was considered positive or negative when its mean A_{492} was higher or lower (equal) than the cut-off value, respectively. The obtained data were analysed with Student's t-test and subjected to correlation analysis.

Results

In the initial screening, the sera from 20 individuals immunized with ICV against Q fever and from 10 subjects injected with PBS (placebo) were tested for the presence of antibodies against *C.b.* (Table 1). According to CFT, only 40% of the subjects had antibodies to *C.b.* after vaccination. The percentage of individuals with specific immune response was higher in IIFT (55%) than in CFT, and also the IIFT titers were higher than the CFT ones. The serum antibody titers in different subjects reached maximum level at a distinct period of the observation and there was also some diversity in the dynamics of antibody production in different individuals.

For the detection of antibodies against *C.b.*, we also used ELISA in which the plate wells were coated with HCl-hydrolysed corpuscular antigen of phase I *C.b.*. As revealed by ELISA, 80% of sera of vaccinees contained the antibodies against *C.b.* and the level of antibody titers in some

Table 1. Activity of anti-*C.b.* antibodies in the sera of individuals immunized with ICV against Q fever according to three different tests

Serum No.	Antibody titers											
	CFT Days a.i.				IIFT Days a.i.				ELISA Days a.i.			
	14	21	28	60	14	21	28	60	14	21	28	60
2	0	0	0	0	0	0	0	0	0	200	200	200
3	0	0	0	0	0	0	0	0	400	400	400	200
4	0	0	0	0	0	0	0	0	200	200	200	400
5	0	0	10	20	0	16	64	32	800	800	800	800
6	0	80	80	160	256	1024	2048	2048	800	3200	3200	800
8	0	0	0	0	0	0	0	0	800	3200	3200	400
9	0	40	160	160	512	512	512	1024	800	3200	3200	400
10	0	0	0	0	0	0	0	64	800	800	800	3200
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	400
17	0	0	0	0	0	8	32	8	200	200	200	800
18	0	0	0	80	32	256	256	512	800	1600	1600	3200
19	40	160	ND	320	256	1024	ND	512	12800	25600	ND	25600
20	0	0	0	0	0	0	0	8	800	800	800	400
21	10	ND	10	80	32	ND	256	64	400	ND	400	3200
23	0	0	0	0	0	0	0	0	0	0	0	0
24	10	20	40	80	8	512	1024	1024	6400	6400	6400	12800
25	10	20	20	20	0	64	256	64	6400	6400	6400	3200
27	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0
Positive reactions (%)	15	25	30	40	20	40	40	55	70	75	75	80

ND = not done; a.i. = after immunization.

subjects remained high till the end of the observation period. The increased percentage of sera with specific antibodies in ELISA is the result of a higher sensitivity of this method than that of CFT or IIFT. ELISA has been shown to increase the antibodies detection sensitivity in comparison with CFT by 100-fold (antibody titers in CFT 1:10-1:320) and in comparison with IIFT by 10-fold (antibody titers in IIFT 1:8-1:2048). All sera of the subjects of the control (placebo) group were negative either in ELISA or in other tests during the whole observation period. Among the immunized subjects with a specific immune response detected in ELISA, there was a group of strongly reacting individuals (9) with the titers of anti-*C.b.* antibodies equal to or above 1:3200.

To determine whether the immunization of individuals with vaccine against Q fever had induced an autoantibody response, sera of 20 immunized subjects and 10 individuals of the control group were assayed by ELISA for binding to soluble antigens. Using the cut-off value described above, it was shown that none of the subjects that received PBS (placebo) exhibited a significant increase of reactivity with any of the antigens used ($P > 0.05$). By contrast, it was found that in comparison with the control group the vaccinated individuals tended to show an increased autoantibody activity to diverse antigens (Fig. 1). The sera of different individuals exhibited a variable degree of binding the antigens. Forty % of sera of the immunized subjects exhibited an anti-Fab activity, 20% of the sera recognized IGA, $F(ab')_2$- and Fc-fragments of IgG, and 15% of the sera recognized IgG anf IgM. To rule out the possibility that the observed reactivity was due to the presence of polyreactive IgM antibodies of the preimmune repertoire, we determined the isotype of the reactive antibodies and found that the induced autoantibodies had different isotypic specificities (IgA, IgG and IgM). The isotypic distribution of autoantibodies against distinct antigens is shown in Fig. 1, which demonstrates a wide variation in the level of autoantibodies and their isotypic heterogeneity. It is interesting to note that the observed anti-Fab antibodies were essentially of the IgA and IgG ($P < 0.05$), but not IgM isotype ($P > 0.05$).

The dynamics of the anti-Fab activity of the sera of one vaccinee before and during 60 days after immunization with vaccine against Q fever is represented in Fig. 2. Here, the

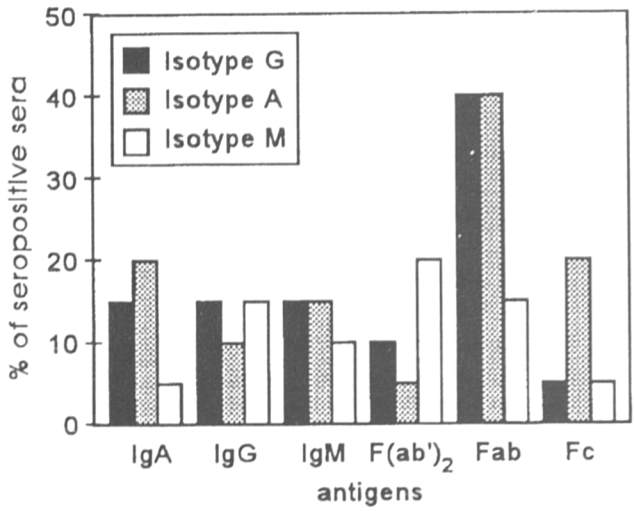

Fig. 1
Autoantibody reactivity of the sera of vaccinees with immunoglobulins and their fragments

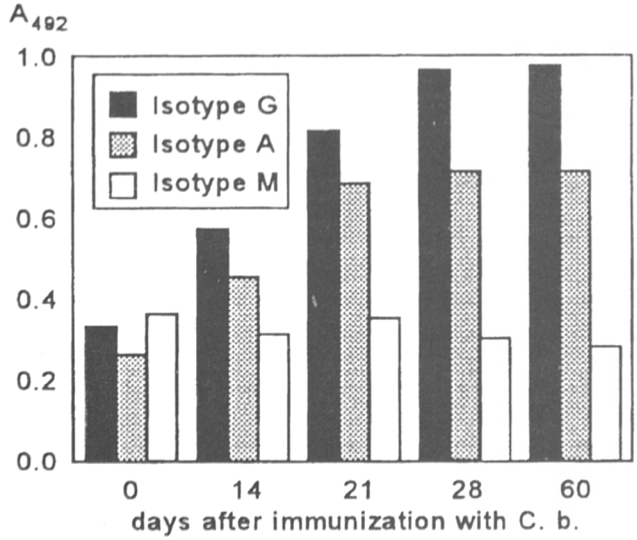

Fig. 2
Anti-Fab reactivity dynamics in the sera of one immunized individual

immunization greatly increased the level of anti-Fab IgG and IgA activities at all intervals as compared to those before immunization, and it did not result in the change of the anti-Fab IgM activity. The sera of other immunized subjects demonstrated a variability with regard to the binding to the Fab-fragment of IgG. It should be emphasised that the highest anti-Fab activity was found in the sera of subjects with high anti-*C.b.* antibody titers.

To look for the correlation of the occurence of autoantibodies with the level of specific immune response in the sera of the vaccinees, were employed the correlation analysis. A significant direct correlation between the level of anti-*C.b.* antibodies and the anti-Fab IgG activity ($r = 0.623$; $P = 0.003$) was found only on the day 60 after immunization. A less significant correlation was detected between the specific antibody response and the anti-Fab IgA activity ($r = 0.378$; $P = 0.05$) also on the day 60 after immunization only. In the group of strongly reacting vaccinees, this tendency increased: correlation became more significant for both the anti-Fab IgG ($r = 0.792$; $P = 0.009$) and the anti-Fab IgA antibodies ($r = 0.623$; $P = 0.05$). No correlation was found with the sera of the placebo group.

Discussion

Autoantibodies, although often associated with autoimmune diseases, have recently been observed in both humans and animals during infections, subsequent to vaccination and during conventional immune responses ((Rodriquez, 1989; Schatner, 1990; Susal, 1992). We confirmed this observation by demonstrating the occurence of autoantibodies against immunoglobulins A, M and G, and their fragments in humans immunized with influenza vaccine or staphylococcus anatoxin (Zhebrun *et al.*, 1991), and in rabbits immunized with bacterial antigens (Krutitskaya, 1988).

In this report, we attempted to find the answer to the question, whether the immunization with vaccine against Q fever could induce an autoantibody production to the same antigens. We showed that the injection of this vaccine can trigger also an autoantibody response to immunoglobulins and their fragments. Using a sensitive ELISA we found that the vaccinees exhibited increased autoantibodies against diverse antigens – IgA, IgM, IgG, $F(ab')_2$, and Fab- and Fc-fragments of IgG. Furthermore, the observed isotypic heterogeneity of these autoantibodies could reflect the presence of multiple antibody populations specific for each antigen. The levels of the induced autoantibodies were markedly higher against Fab-fragments of IgG than against the other examined antigens. The sera of 40% of the immunized individuals contained antibodies against Fab-fragments of IgG. This fact indicated that such antibodies were a normal constituent of serum immunoglobulin pool of immunized individuals. It should be note that the anti-Fab activity of the sera of vaccinees were essentially of IgG and IgA ($P < 0.05$) but not of IgM isotype ($P > 0.05$). By contrast, the subjects of the control (placebo) group did not exhibit significant increase of reactivity with any of the tested antigens ($P > 0.05$).

In addition, the significant correlation between the intensity of the specific immune response and the level of the auto-anti-Fab IgG and IgA activities after vaccination was found. This tendency increased in the group of individuals strongly reacting to the vaccine. At the same time, no such

a correlation was found in the control (placebo) group. These results are in agreement with those from our previous study, which demonstrated the induction of autoantibodies to Fab- and other fragments of IgG in individuals immunized with influenza vaccine (Krutitskaya, 1992). As it has been shown, the immunization with influenza vaccine induced isotypic heterogenous autoimmune reactions during the six months of the observation, and moreover, the existence of direct correlation between the intensity of specific immune response and the autoantibody reactivity with the antigen binding fragments has been revealed too.

The reason why the autoantibodies against immunoglobulins and their fragments are produced during the immune response has not been elucidated. The activation of the autoantibody production after immunization is probably induced by the manifestation of a specific immune response to foreign antigens. We can just speculate that the immune complexes formed during the immune response in humans or animals could be responsible for stimulation of the autoantibody production. However, the autoantibodies against the fragments of Ig, that we detected, may be at least partially directed toward "hidden" determinants that are exposed after IgG digestion. We suggest that the autoantibodies induced by immunization are formed as a part of a physiological mechanism participating in the processes of immune complex catabolism.

The observation that the sera of individuals immunized with different antigens contained anti-Fab antibodies implies that the induction of auto-anti-Fab activity is a steady-state process, and leads us to suppose a regulatory function of these antibodies, especially in the light of the fact that this activity was directed toward the Fab region of the IgG molecule. It is unlikely that the interactions of anti-Fab IgG and anti-Fab IgA are allotype- or idiotype-mediated, because, as we described earlier (Zhebrun, 1991), the induced antibodies bind to Fab-fragments of the autologous IgG as well as to a variety of homologous IgG. Moreover, unlike the anti-idiotypic reactions, these autoimmune reactions were not antigen-specific, because the autoantibodies of the same specificity after immunization with different antigens were revealed. We suppose that the autoantibody production during immune response to different antigens is a physiological phenomenon, and the decreased levels of these autoantibodies in some individuals with weakly specific immune response after immunization may account for some immunoregulatory defects.

References

Bodansky HJ, Dean BM, McNally J (1989): Islet cell and other autoantibody responses to rubella vaccination. *Diabetologia* **32**, 468–471.

Brock J (1987): Isolation of immunoglobulins. In Friemel H (Ed.): *Immunological Methods.* Moscow, pp. 390–396, 421–423 (in Russian).

Gorbachev EN, Tokarevich NK, Fiodorov DK (1989): Detection of the antibodies to *Coxiella burnetii* antigens in ELISA. In Dayter AB, Rzhegachek I (Ed.): *Rickettsioses.* Leningrad, pp. 127–136 (in Russian).

Jehanly A, Hough D (1981): A rapid procedure for the isolation of human IgM myeloma proteins. *J. Immunol. Meth.* **44**, 199–204.

Kováčová E, Gallo J, Schramek S (1987): Comparison of the natural and chemicaly treated *Coxiella burnetii* corpuscular antigens in the determination of the antibodies to Q fever in human sera with using ELISA. *Acta Virol.* **31**, 254–259.

Krutitskaya LI, Polosatov MV (1988): The nature of serum factor precipitating the autologous a-globulins in rabbits. *Bull. Exp. Biol. Med.* **9**, 331–334 (in Russian).

Krutitskaya LI, Zhebrun AB (1992): Isotypic distribution of the autoantibodies to IgG and their fragments in individuals immunized with influenca vaccine and stafylococus anatoxin. In Petrov RV (Ed.): *Ith Symposium of Russian Immunologists.* Novosibirsk, pp. 225 (in Russian).

Porter PR (1959):The hydrolysis of g-globulin and antibodies with crystalline papain. *Biochem. J.* **73**, 119–124.

Rodriquez MA, Williams RC (1989): Infection and rheumatic diseases. *Clin. Exp. Rheumatol.* **7**, 91–96.

Schatner A, Rager-Zisman B (1990): Virus-induced autoimmunity. *Rev. Infect. Dis.* **12**, 204–222.

Susal C, Lewin IV, Stanworth DR (1992): Anti-IgG autoantibodies in HIV infected hemophilia patients. *Vox. Sang.* **62**, 224–229.

Tarasevich I, Yablonskaya V, Fatalieva S, Uskov V, Dayter A, Tokarevich N (1995): Inactivated combinated vaccine from *Coxiella burnetii* phase I against Q fever. *Proc. Meet. Am. Soc. Rickettsiol. Rickettsial Dis.* September 17-20, 1995, St. Simons Island, Georgia, USA.

Welch MJ, Fong S, Vaughan J (1983): Increased frequency of rheumatoid factor precursor B lymphocytes after immunization of normal adults with tetanus toxoid. *Clin. Exp. Immunol.* **51**, 299–305.

Yablonskaya VA, Tarasevich IV, Phatalieva SP (1994): Results of the study of inactivated combinated vaccine from *Coxiella burnetii* phase I against Q fever. In Tarasevich (Ed.): *Problems of Rickettsiology.* Moscow, pp. 114–138 (in Russian).

Zhebrun AB, Krutitskaya LI (1991): Autoimmune reactions during experimental immunization and vaccination of donors. In Kalinin YT, Kurstak E (Eds): *Proc. Int. Conf. Med. Biotechnol. Immun. AIDS.* Leningrad, S3–7 (in Russian).

Zhebrun AB, Krutitskaya LI (1992): Development and analysis of human IgA, IgM, IgG. In Glinskich AP (Ed.): *Epidemiology Clinic and Prophylaxis of Virus Infections.* Ekaterinburg, pp. 11–17 (in Russian).