Vaccination of healthy subjects and autoantibodies: from mice through dogs to humans

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Vaccination against pathogenic microorganisms is one of the major achievements of modern medicine, but due to an increasing number of reports of adverse reactions the vaccination procedure has induced also considerable debate. It is well known that certain infections are involved in triggering the production of autoantibodies, which could lead to autoimmune adverse reactions in genetically predisposed subjects. Based on these findings it was assumed that vaccinations might induce similar autoimmune reactions. At present there is no clear-cut evidence that vaccinations are associated with overt autoimmune diseases but it has been demonstrated that in genetically predisposed persons vaccination can trigger the production of autoantibodies and autoimmune adverse reactions. The first studies investigating the production of autoantibodies following vaccination were done in dogs and mice. Several studies investigated the production of autoantibodies following vaccination in patients with autoimmune diseases, but there are only limited data on the autoimmune responses after vaccinations in apparently healthy humans. This review summarizes current evidence on the vaccination-induced autoantibodies in apparently healthy subjects including studies in animals and humans. Lupus (2009) 18, 1186–1191.

Key words: antiphospholipid antibodies; autoantibodies; healthy subjects; vaccination

Introduction

Autoantibodies are a hallmark of autoimmune diseases but can also be positive in apparently healthy individuals, mainly in elderly people. Several environmental triggers could induce production of autoantibodies which may appear transiently or persistently, and conceivably lead to the development of an overt autoimmune disease. Infections are one of the most compelling environmental triggers for autoimmunity in persons with genetic susceptibility. It has been shown in several studies that infections can induce or exacerbate autoimmune diseases or trigger transient production of autoantibodies without clinical significance.1,2

One of the possible environmental triggers for inducing the production of autoantibodies is also vaccination. It has been assumed that vaccinations can trigger the same autoimmune reactions as infections in genetically predisposed persons.3 However, this assumption may be contradictory in view of the fact that vaccinations essentially prevent infections. Currently, there is no scientific evidence that vaccinations can be directly associated with the development of autoimmune diseases, but several isolated reports exist about autoimmune adverse reactions following various vaccinations.1,3 A transient production of autoantibodies following vaccination without obvious clinical significance has been described in patients with different autoimmune diseases but only scarce data exist in healthy persons. This review summarizes current evidence on the vaccination-induced autoantibodies in apparently healthy subjects including studies in animals and humans.

Autoantibodies in healthy subjects

It is widely known that autoantibodies may be detected in a certain percentage of apparently
healthy subjects. An autoimmune response can be evoked by natural antibodies that are able to bind to microbial antigens, altered proteins and self-antigens. Natural autoantibodies possess in general a low intrinsic affinity for antigen, but can function as templates for the generation of pathogenic autoantibodies.4

Scarce information is available regarding autoantibodies in healthy animals. In a study of canine atopic dermatitis none of 20 apparently healthy dogs in a control group were positive for antinuclear antigen antibodies (ANAs).5 Papini et al. demonstrated that anticardiolipin antibodies (aCL) are more frequent in older than in younger dogs.6

The frequency of ANAs in the healthy human population varies with age, gender, ethnic origin, environmental exposures and laboratory method used to detect them.7 Several studies investigated the presence of ANAs in humans. Hilario et al. found a positive ANAs titer equal to or higher than 1:80 in 12.6% of healthy children aged 6 months to 20 years with no significant difference between genders. Eight of the 27 healthy children with positive ANAs were reevaluated 36 months later and two of them remained positive with no apparent autoimmune disease.7 In a retrospective study of 110 children referred to the Rheumatology Clinic because of a positive ANAs test, McGhee et al. reported that 80 children in this group did not have a chronic inflammatory disorder.8

Tan et al. studied apparently healthy adults and found that 31.7% were positive for ANAs at a 1:40 dilution, 13.3% at 1:80, 5% at 1:160 and 3.3% at 1:320 dilutions, respectively.9

In a study of ANA prevalence among first-degree relatives and spouses of systemic lupus erythematosus (SLE) patients, ANA positivity was found in 31% at 1:40 dilution, 24% at 1:80 and 10% at 1:160 dilution in first-degree relatives. Among spouses, 10% was positive at 1:40 dilution, 4% at 1:80 and none at 1:160. In a control group of healthy blood donors the percentages of ANA-positive persons was almost the same as among spouses. According to these results it seems that inherited genetic factors are responsible for the development of ANAs in first-degree relatives of SLE patients and that effect of shared environment in adult life on factors determining ANA prevalence is negligible. This study also assessed the antiphospholipid antibodies (aPLs). Among first degree relatives 6% were found to have aCL and 0.4% anti-β2-glycoprotein I (anti-β2-GPI) antibodies. Among spouses 4% had aCL and none anti-β2-GPI. The occurrence of aPLs did not differ between first-degree relatives, spouses and the healthy blood donor group.10

As with most other autoantibodies, an increased frequency of aPLs has been reported in the healthy elderly population compared with healthy adults. It is generally assumed that this autoimmune phenomenon might be a manifestation of age related senescence of the immune system.11 One of the characteristics of the aged immune system is a loss of the ability to recognize self- and foreign antigens and the development of autoantibodies secondary to the thymus involution with a decline of naive T-cells an accumulation of clonal T-cells.12 There is also some recent evidence for possible autoimmune responses of the developing immune system during childhood, which was demonstrated in a study of 61 apparently healthy children that exhibited the frequency of aCL similar to values obtained in healthy adults.13

There are probably several reasons for the phenomenon of the presence of autoantibodies in apparently healthy subjects. In particular, transiently elevated autoantibodies could be related to a preceding acute infection and/or vaccination. A fundamental question which remains unclear is the clinical and biological significance of the mainly low-titer antibodies that are present in apparently healthy population.

Vaccination and autoantibodies in healthy subjects

Scarce information exists about autoimmune responses following routine vaccinations in healthy subjects and only recently have a few studies been published investigating the production of autoantibodies in apparently healthy adults. To the best of the authors’ knowledge, there were no published studies investigating the production of autoantibodies following routine vaccinations in healthy children.

The first controlled experimental study investigating production of autoantibodies following routine vaccination was conducted in dogs in the late 1990s.14 Hogenesch et al. found that mandatory vaccination against rabies, canine distemper virus and canine parvovirus triggered the production of various autoantibodies. The reason for this study was the frequent complaints of dog owners after mandatory vaccinations of their dogs. Many of them reported that their dogs became ill after vaccination. The study assessed the effect of
vaccination in young dogs that had not been immunized previously. The dogs in a study group were immunized with commercially available multivalent vaccine at 8, 10, 12, 16 and 20 weeks of age. The control group received subcutaneous injections of sterile saline at the same time points. Two weeks after the last vaccination there was an increase in the titer of IgG antibodies reactive with 10 of 17 antigens in a study group. A significant increase was observed for antibodies against laminin and fibronectin. No increase was observed in a control group of non-vaccinated dogs. The study was terminated 2 weeks after the last vaccination and at this time there were no signs of autoimmune diseases in the dogs. Another study investigated the production of antithyroglobulin antibodies after routine vaccination in pet and research dogs. Researches found a significant increase in anticanine thyroglobulin antibodies in only two groups of dogs that received the rabies vaccine.

In 2002, Blank et al. provided first experimental evidence for infectious origin of antiphospholipid syndrome (APS). Mice immunized with a panel of microbial preparations were studied for the development of anti-β2-GPI antibodies. Pathogenic anti-β2-GPI antibodies directed against the hexapeptide TLRVYK epitope were synthesized in mice that were immunized with Haemophilus influenzae or Neisseria Gonorrhoeae, which both exhibit the TLRVYK sequence. Pathogenic anti-β2-GPI antibodies were also formed in mice immunized with tetanus toxoid, which does not present the linear TLRVYK sequence but conformationally mimics it. In this experiment, the pathogenic anti-β2-GPI antibodies were purified from the immunized mice and passively infused into naive mice at the very beginning of the pregnancy. APS parameters were evaluated in the infused mice on day 15 of pregnancy. Significant thrombocytopenia, prolonged activated partial thromboplastin time and elevated percentage of foetal loss were found in infused mice. This study established a mechanism of molecular mimicry in experimental APS, demonstrating that bacterial peptides homologous with β2-GPI induce pathogenic anti-β2-GPI antibodies along with APS manifestations.

Recently, another study that focused on tetanus toxoid as a model for microbial antigen was published. Non-pretreated and complete Freund's adjuvant pretreated BALB mice were immunized with high doses of tetanus toxoid mixed with glyceral or aluminium hydroxide as adjuvants. Immunization was performed three times at 2-week intervals. Sixteen weeks after the third dose a booster dose with appropriate adjuvant was administered. Samples of sera were collected before immunization, 1 week after the third immunization, 11 weeks after the third immunization and 1 week after the booster tetanus toxoid dose application. Detection of antibodies specific for tetanus toxoid, β2-GPI and laminin were performed. There were no significant time-dependent changes in mean concentrations of tetanus toxoid antibodies and only slight fluctuations within the anti-laminin IgG pool were detected in the study subgroups, except in the subgroup of Freund's adjuvant pretreated mice immunized with high doses of tetanus toxoid mixed with aluminium hydroxide as adjuvant. In this group the concentrations of anti-laminin IgG found 1 week after completion of immunization was significantly higher than that in normal sera. Preliminary screening for IgM and IgG anti-β2-GPI antibodies showed the presence of these antibodies but neither pretreatment nor adjuvants per se induced increase in their level. Subclass analysis and affinity of anti-β2-GPI IgG were tested. A statistically significant difference in dissociation profiles between identically pretreated groups was registered only after complete Freund adjuvant pretreatment. The analysis of pregnancy outcomes following tetanus toxoid immunization was used for the evaluation of the pathogenic potential of induced immune responses, associated with anti-β2-GPI antibodies. It was demonstrated that the severity of pathology was positively correlated to the abundance of IgG that recognizes β2-GPI adsorbed on phosphatidylserine, and to IgG affinity. Autoimmunity can also be triggered by adjuvant hydrocarbons. Lupus-like disease with disease-specific autoantibodies and nephritis developed following pristan injection in non-autoimmune prone mice. Some strains also developed an erosive and destructive arthritis. Moreover, it was shown that other hydrocarbons, notably the mineral oil Bayol F and the endogenous hydrocarbon squalen, can induce lupus-like disease in mice. Adjuvants have been added in human vaccines to boost the immune response. At present, only a few effective adjuvants are considered safe for use in humans.

The production of autoantibodies following vaccinations with recombinant hepatitis B and influenza vaccine in humans was recently investigated in two studies. Both studies were completed after 6 months and autoantibodies were determined before, 1 and 6 months after vaccination. The induction of aPLs following vaccination with recombinant DNA hepatitis B vaccine was investigated in 85 healthy volunteers who were medical
A transient increase of aCL titers in two participants and a transient increase of anti-β2-GPI titers in one participant were observed. One participant who initially had low positive IgG anti-β2-GPI showed a progressive increase of the antibody level during 6 months of follow up. There was no statistically significant production of aPLs after vaccination with hepatitis B vaccine in healthy adults but long-term aPL response in genetically predisposed individuals could not be excluded.19

In the study of autoimmune response following influenza vaccination the study group consisted of 92 apparently healthy medical workers at the University Children’s Hospital Ljubljana.20 A high percentage of positive autoantibodies were observed already before the influenza vaccination including 26% of participants with positive ANA at the cut off value of 1:80 or higher, 16% with positive aCL, 7% with positive anti-β2-GPI, 2% with positive lupus anticoagulant (LA) and 1% with positive antibodies against extractable nuclear antigens (anti-ENA). These high percentages of autoantibodies detected before vaccination in healthy medical workers were not readily explained, but may be associated with a higher exposure to environmental factors such as certain infectious agents. Sixty-two per cent of participants had protective antibodies to at least one strain of the influenza virus before the vaccination. However, there were no correlation between the presence of autoantibodies and protective antibodies against the three different strains of the influenza virus before vaccination. Overall, this study demonstrated no statistically significant difference in the percentage of participants with positive autoantibodies before, 1 and 6 months after the influenza vaccination. Increased level of autoantibodies or appearance of new autoantibodies was observed 1 month after the vaccination in 15% and 6 months after the vaccination in 13% of participants, suggesting de novo induction of autoantibodies after the influenza vaccination in selected individuals. Changes in the level of autoantibodies were most frequently observed for aCL and anti-β2-GPI antibodies. Two participants demonstrated progressive increase in autoantibody levels including one with increasing levels of IgM aCL and one with increasing levels of IgA anti-β2-GPI antibodies, respectively. No participant developed clinical signs of overt autoimmune disease and no participant demonstrated aPL-related thrombotic events during the 6-month follow-up period after the influenza vaccination.20

Vaccination and autoimmune adverse reactions in healthy subjects

It is now known that vaccinations in healthy subjects can rarely induce the production of autoantibodies that usually appear transiently and in low titers. However, the question of triggering an overt autoimmune disease following vaccination remains to be clarified. Several reports of autoimmune adverse reactions have been published following various vaccinations (Table 1).1,3,21 The majority of epidemiological studies published so far did not confirm an association between vaccination and autoimmune diseases. The autoimmune reaction was usually transient. Hepatitis B vaccine has been connected with multiple sclerosis, rheumatoid arthritis, reactive arthritis, SLE, polymyositis/dermatomyositis, polyarteritis nodosa and idiopathic thrombocytopenia.3,22 According to the Vaccine Adverse Event Reporting System (VAERS), Guillain–Barré

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<th>Autoimmune adverse reaction/autoimmune diseases</th>
<th>Vaccines</th>
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<tr>
<td>Arthritis (reactive or rheumatoid)</td>
<td>Tetanus, diphtheria, polio, measles, mumps, rubella, hepatitis B, influenza</td>
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<tr>
<td>Systemic lupus erythematosus</td>
<td>Tetanus, hepatitis B</td>
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<tr>
<td>Polymiositis/dermatomyositis</td>
<td>Tetanus, diphtheria, hepatitis B</td>
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<tr>
<td>Guillain–Barré syndrome</td>
<td>Tetanus, diphtheria, polio, rubella, mumps, hepatitis B, bacillus, Calmette–Guérin, influenza, rabies</td>
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<tr>
<td>Multiple sclerosis</td>
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<td>Diabetes mellitus type 1</td>
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<tr>
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<td>Henoch–Schönlein purpura</td>
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<td>Idiopathic thrombocytopenia</td>
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Table 1 Autoimmune adverse reactions and autoimmune diseases reported following vaccinations in apparently healthy humans1,3,21–25
syndrome represents the most frequently reported autoimmune neurological adverse event following influenza vaccination. A few cases of Henoch–Schönlein purpura with induction of aPLs have been described in children following influenza vaccination. To the best of authors’ knowledge, there are no published reports of APS following vaccinations in healthy subjects.

**Pathogenesis of autoantibodies production following vaccination**

Several mechanisms that could be involved in autoimmunity following infection or vaccination have been proposed. Much attention has been focused on the mechanisms of B- and T-cells tolerance to self-antigens; however, less is known about the role of the innate immune system in autoimmune processes. The production of autoantibodies and autoreactive T-cells may rely on the continued presence of infectious organisms or its residual antigen and it is possible that antigenic determinants of vaccines or residues contain a sequence similar to a self-antigen to produce cross-reactivity by the mechanism of molecular mimicry. However, the involvement of self-antigens cannot be excluded. Possible mechanisms include epitope spreading, polyclonal activation of T- or B-cells by mitogens of the invading organism and loss of downregulation that occurs within dendritic cells (DCs) clusters. It has been assumed that DCs as the professional antigen presenting cells (APCs) play one of the major roles in priming of autoimmune processes. Toll-like receptors (TLRs) on DCs and other cells of innate immunity bind conserved microbial products. It is however interesting that TLRs can also be found on B-cells. Actually, B-cells may act as a bridge between innate and adaptive immunity since they can be directly activated through TLRs and indirectly by interferon α (IFN α). B-cells have a more central role in the maintenance of the autoimmune disease process than just being the precursors of autoantibody producing plasma cells. In mice, but not yet in humans, a regulatory B-cells (Breg) have been identified that have the capacity to restrain immune responses and thus prevent pathogenic autoimmunity. Defective Breg cell function could contribute to the development of human autoimmune diseases due to faulty control either through their interleukin-10 mediated effects or through direct cellular interaction.

It has been shown by Heer et al. in evaluating the role of innate signals on the quality of the anti-influenza immune response in vivo that effective T-cell response against influenza does not require T-cell signalling. TLR signalling influences the B-cell response directly. This might have a role in infection. In vaccinations against viral diseases, such as influenza, no genetic material is present so direct induction of B-cells through TLRs does not play a role. Another possibility of induction of B-cells is indirectly through IFN α induction and this mechanism could play a role in autoimmune response following infection and also vaccination. Several cytokines are potentially released following immunization and therapeutic use of IFN α was associated with induction or worsening of autoimmune diseases.

As we have already described, experimental data supporting the mechanism of vaccination-induced autoantibodies have been demonstrated by Blank et al. Pathogenic anti-β2-GPI, directed against hexapeptide TLRVYK, were synthesized in mice following immunization with *H. influenzae*, *N. Gonorrhoeae* and tetanus toxoid. It is plausible to hypothesize that similar mechanisms may be involved in triggering the production of aPLs after vaccinations in humans. Actually, it cannot be excluded that some positive values of aPLs detected in apparently healthy children could be the result of previous infections and/or vaccinations particularly against tetanus and *H. influenzae*.

**Conclusions**

Current evidence suggests that vaccinations of healthy subjects may seldom induce the production of autoantibodies that usually appear transiently and in low titres. The association has been suggested mainly for aPL, in particular anti-β2-GPI antibodies, following immunization of mice with *H. influenzae* and tetanus toxoid, and in humans following vaccinations against hepatitis B and influenza. The mechanism of molecular mimicry in induction of anti-β2-GPI antibodies following infection and vaccination has been shown in the model of experimental APS in mice. The issue of triggering an overt chronic autoimmune disease following various vaccinations remains controversial and needs to be studied in larger, prospective studies with long observation time following vaccination. For the present vaccination practice it certainly appears that the benefits of vaccination...
References


