ORIGINAL ARTICLE

# Autoimmunity and Hepatitis A Vaccine in Children

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■ Abstract

*Background:* Universal vaccination remains the most effective way of preventing the spread of many infectious diseases. Although most adverse effects attributed to vaccines are mild, rare reactions such as autoimmunity do occur.
*Objectives:* We aimed to evaluate the possible role played by hepatitis A vaccine (HAV) in inducing the synthesis of autoantibodies. The study included 40 healthy children vaccinated with 2 doses of HAV at a 6-month interval. The children were investigated for autoantibodies including anti-nuclear antibodies (ANAs), anti–smooth muscle antibodies, anti–nDNA, anti–microsomal antibodies, anti-cardiolipin (aCL) immunoglobulin (Ig) M/IgG, anti-ds DNA, ANA profile, and anti–neutrophil cytoplasmic antibody profile.
*Results:* One month after the first dose, ANAs at a titer of 1:100 and aCL IgG at 23.7 IgM phospholipid units were detected in 4 children and 1 child, respectively. Of the ANA-positive children, 1 also had ASMA positivity, and another had perinuclear and cytoplasmic ANCA positivity. After the second dose, 3 of the children had aCL IgM. In addition, 2 distinct children had positive anti-thyroid microsomal antibodies and ANA after the second dose. The presence of these autoantibodies following vaccination was statistically significant ($P=.002$). At month 12 of the study, only 2 children continued to be ANA-positive at the same titer as after the first vaccine dose.
*Conclusions:* Although HAV can induce the production of autoantibodies, none of the children developed autoimmune disorders. Long-term follow up is necessary to check whether autoimmune disorders develop in children who still have ANA. Genetic, immunological, environmental, and hormonal factors are also important in the development of vaccine-induced autoimmunity.

Key words: Hepatitis A vaccine. Autoimmunity. ANA. Anti-cardiolipin antibody.

■ Resumen

*Antecedentes:* La vacunación universal sigue siendo la manera más eficaz de prevenir la propagación de muchas enfermedades infecciosas. Si bien la mayoría de los efectos adversos atribuidos a las vacunas son leves, en ocasiones se producen reacciones poco frecuentes como la autoinmunidad.
*Objetivos:* Evaluar el posible papel de la vacuna de la hepatitis A (VHA) en la inducción de la síntesis de autoanticuerpos. En el estudio se incluyó a 40 niños sanos vacunados con 2 dosis de VHA con un intervalo de 6 meses. Se estudió la presencia de autoanticuerpos en los niños, como anticuerpos antinucleares (ANA), anticuerpos antimúsculo liso (ASMA), anticuerpos anti ADNn, anticuerpos antimicrosómicos, inmunoglobulina (Ig) M/IgG anticardiolipina (aCL), anti ADNds, así como el perfil de ANA y el perfil de anticuerpos anticitoplasma de neutrófilos (ANCA).
*Resultados:* Un mes después de la primera dosis, se detectaron ANA con un título de 1:100 en 4 niños e IgG aCL con un título de 23,7 unidades de fosfolípidos IgM en 1 niño. De los niños positivos para ANA, uno también resultó positivo para ASMA, y otro resultó positivo para ANCA perinucleares y citoplásmicos. Después de la segunda dosis, 3 niños presentaban IgM aCL. Asimismo, 2 niños distintos fueron positivos para anticuerpos microsómicos antitiroideos y ANA tras la segunda dosis. La presencia de estos autoanticuerpos después de la vacunación fue estadísticamente significativa ($p = 0,002$). En el mes 12 del estudio, solo 2 niños seguían siendo positivos para ANA con el mismo título que después de la primera dosis de la vacuna.
*Conclusiones:* Aunque la VHA puede inducir la producción de autoanticuerpos, ningún niño desarrolló trastornos autoinmunitarios. Es necesario realizar un seguimiento a largo plazo para comprobar si los niños que todavía presentan ANA desarrollan trastornos autoinmunitarios. Los factores genéticos, inmunológicos, ambientales y hormonales también son importantes en el desarrollo de autoinmunidad inducida por vacunas.

Palabras clave: Vacuna de la hepatitis A. Autoinmunidad. ANA. Anticuerpo anticardiolipina.

## Introduction

Vaccines have been used since 1798 and are the most effective way of preventing morbidity and mortality associated with infections. Since vaccination is administered to healthy individuals, safety is primordial. Several case reports and studies have reported the appearance of autoantibodies and autoimmune phenomena such as vasculitis, neuropathy, and demyelinating disease following vaccination [1]. There are no established criteria for diagnosing vaccine-related autoimmune disease. Postvaccination autoimmunity occurs quite a long time after vaccination, making it difficult to ascertain causality [1,2]. Vaccines are composed of infectious agents, adjuvants, preservatives, and other ingredients. Each of these components might induce an immune response, which, in turn, could induce or aggravate autoimmunity. Infectious agents stimulate autoimmunity via molecular mimicry, epitope spread, bystander activation, and polyclonal activation [3]. Antigenic similarity between microbial molecules and host antigens is an important factor. One third of all cases of Guillain Barre syndrome, for example, are preceded by *Campylobacter jejuni* infection. This bacterium has a lipopolysaccharide molecule that mimics various gangliosides present in high concentrations in peripheral nerves [4]. Viral infections are also associated with Guillain Barre syndrome. A causal relationship between the influenza vaccine and Guillain Barre syndrome was also reported following an outbreak of the disease after administration of the swine flu vaccine in 1976 [5].

Adjuvants are used to stimulate the immune system and increase response to vaccines, but they do not have a specific antigenic effect. Examples of adjuvants currently used in human and animal vaccination programs are alum (aluminium salts), virosomes, and oil-based compounds. Novel and experimental adjuvants based on chemical innovations have been developed by pharmaceutical companies. Alum is the most commonly used adjuvant in human vaccines [6], and it has been suggested that macrophagic myofasciitis, a new autoimmune disease, can develop in genetically susceptible individuals following vaccination with alum-containing vaccines [7-9].

There are many claims and counterclaims related to autoimmunity risk following vaccination. In this article, on the basis of published evidence and our own experience, we evaluate the clinical and laboratory findings of children following hepatitis A vaccination to determine the presence of autoantibodies and the development of autoimmune disease. We also discuss various aspects of the causal interactions between vaccines and autoimmune phenomena, as well as possible mechanisms by which different vaccine components might induce autoimmunity.

## Participants and Methods

The study comprised 40 healthy children (10 girls, 30 boys) with a mean age of 6.3 years (range 2-18 years) who visited pediatric immunology outpatient clinics between January 2007 and August 2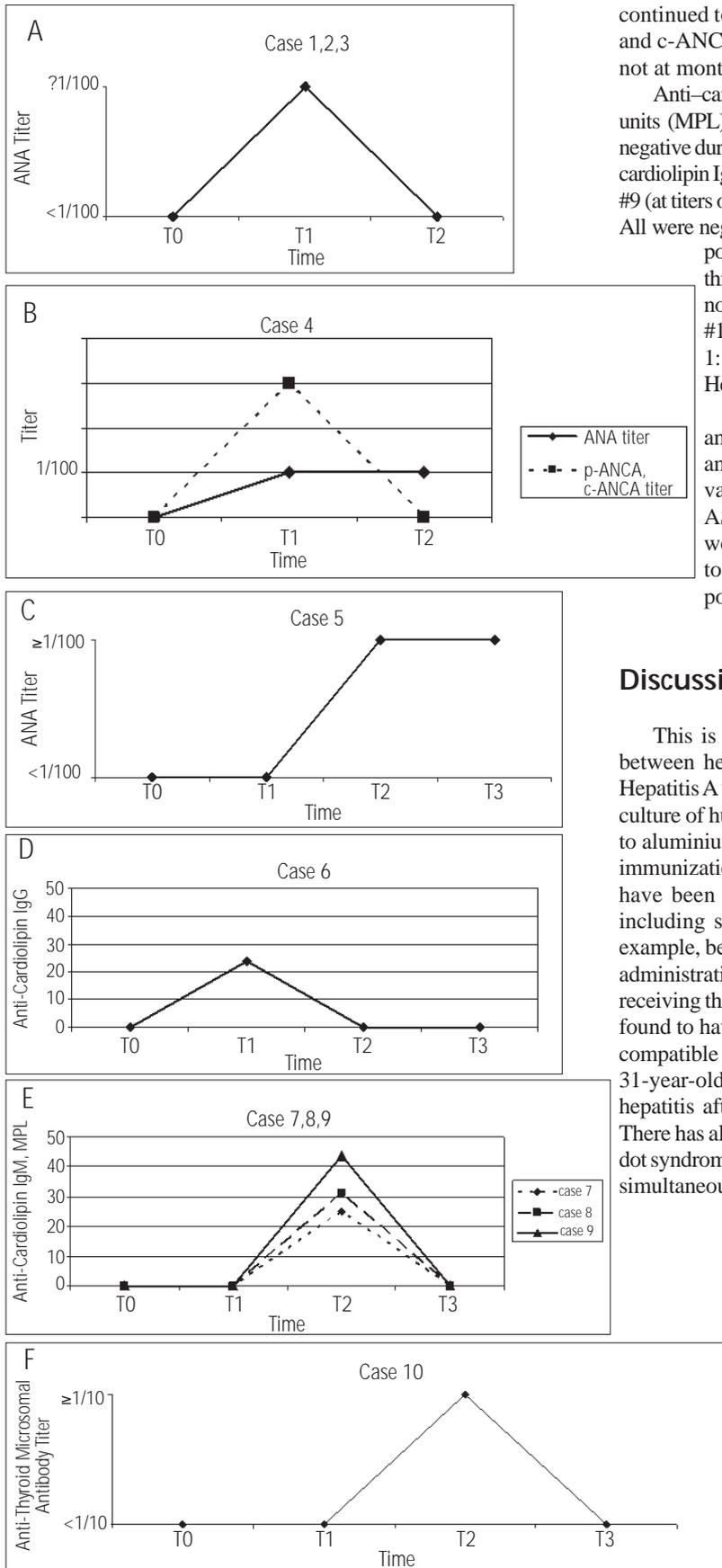009. The children were immunocompetent and negative for hepatitis A immunoglobulin (Ig) M and IgG serology prior to vaccination. The vaccination schedule included 2 doses of hepatitis A vaccine (Havrix 720 mcg/0.5mL, Glaxo Smith Klein) administered 6 months apart.

None of the children had any signs of infection when their blood samples were taken. Before the first vaccine dose and 1 month after the first and second doses, the children were evaluated for complete blood count, liver enzymes (aspartate aminotransferase [AST], alanine transaminase [ALT]), anti–hepatitis A virus IgM/IgG, and autoantibodies including antinuclear antibodies (ANA), anti–smooth muscle antibodies (ASMA), anti–native DNA (nDNA), anti–microsomal antibodies, anti–cardiolipin IgM/IgG, anti-ds DNA, ANA profile and anti–neutrophil cytoplasmic antibody (ANCA) profile (p-ANCA, c-ANCA, MPO). ANA, ASMA, anti-nDNA, ANCA profile and anti–thyroid microsomal antibodies were tested by the immunofluorescent assay (IFA) method (Euroimmune, Lübeck, Germany) and anti–cardiolipin IgM/IgG, anti-ds DNA were tested by enzyme-linked immunosorbent assay (Zeus Scientific, Branchburg, New Jersey, USA). As a substrate for IFA, the ANA test kit contained the human epithelial cell line HEp-20-10, which is of identical genetic origin to HEp-2 cells from human laryngeal carcinoma. According to the recommendations of the manufacturer, an ANA titer of 1:100 and above was considered positive. Thus, in interpreting the results, titers below 1:100 were considered negative, meaning that no antibodies against cell nuclei were detectable in the sample. Rat stomach and animal pathogenic flagellates of *Crithidia luciliae* were used as standard substrates for the detection of ASMA and nDNA, respectively. The cutoff titer for ASMA was 1:100 whereas a titer of above 1:10 was used as a cutoff for ANCA, anti-nDNA, and anti–thyroid microsomal antibody positivity.

Approval for the study was obtained from the ethics committee at Uludag University Medical Faculty and informed consent was obtained from all the children's parents. Statistical analysis was performed by SPSS version 16.0 for Windows. The McNemmar test was used to compare related variables. A $P$ value of <.05 was considered statistically significant.

## Results

All the children had normal complete blood counts and AST and ALT levels, and displayed no antibodies on the screening template before vaccination. The children with negative anti–hepatitis A virus IgG and IgM serology were vaccinated. Four weeks after the first vaccine dose, leukopenia (white blood cell count, 3850/mm$^3$) was detected in 1 of the children and it persisted for 2 weeks. No evidence of infection was detected during this time. During the study period, 10 children showed positivity to 1 or 2 autoantibodies. After the first vaccine dose, 5 children had anti–nuclear autoantibodies at a titer of 1:100, but their ANA profile was negative. ANA positivity had disappeared in 3 children by month 7 of the study (Figure 1a). Of these children, child #1 had ASMA positivity at a titer of 1:100, which was not detected 1 month after the second dose. Children #4 and #5 had ANA positivity at a titer of 1:100 following the first and second vaccine doses, and

A   Case 1,2,3



B   Case 4

ANA titer

p-ANCA, c-ANCA titer



C   Case 5



D   Case 6



E   Case 7,8,9

case 7
case 8
case 9



F   Case 10

IgM indicates immunoglobulin M; MPL, IgM phospholid units.

continued to be positive at month 12 (Figures 1b-c). p-ANCA and c-ANCA were detected in child #4 after the first dose but not at month 7.

Anti–cardiolipin IgG was positive at 23.7 IgM phospholipid units (MPL) in child #6 after the first vaccine dose but became negative during follow-up (Figure 1d). After the second dose, anti–cardiolipin IgM antibody titers were elevated in children #7, #8, and #9 (at titers of 25.1 MPL, 31.1 MPL, and 43.9 MPL, respectively). All were negative a month later (Figure 1e). Children who were positive for aCL antibodies had normal activated partial thromboplastin time, clotting time, and international normalized ratio. After the second vaccine dose, child #10 displayed anti–microsomal antibodies at a titer of 1:10m, but tested negative after a month (Figure 1f). He had normal thyroid function tests.

At the end of the study none of the children with antibody positivity had signs of autoimmune disease, and they all had anti–hepatitis A virus IgG after the vaccine. Comparisons of complete blood counts and AST and ALT values before and after vaccination were statistically insignificant ($P>.05$). With respect to prevaccination status, 10 children had antibody positivity after the 2 vaccination doses ($P=.002$)

## Discussion

This is the first study to evaluate the causal relationship between hepatitis A vaccine and autoimmunity in children. Hepatitis A vaccine is an inactive vaccine which is prepared in a culture of human fibroblasts, inactivated by formalin, adsorbed to aluminium hydroxide adjuvants, and integrated into routine immunization schedules worldwide [10]. Rare adverse effects have been reported after the universal use of the vaccine, including several autoimmune phenomena. There have, for example, been 2 reports of autoimmune hepatitis following the administration of hepatitis A vaccine. In one case, 10 days after receiving the vaccine, a 56-year-old previously healthy man was found to have anti–nuclear antibodies and his liver biopsy was compatible with autoimmune hepatitis[11], and in the other, a 31-year-old woman abruptly developed severe autoimmune hepatitis after hepatitis A and yellow fever vaccination [12]. There has also been a recent report of multiple evanescent white dot syndrome (an inflammatory chorioretinal disorder) following simultaneous hepatitis A and yellow fever vaccination [13].

Figure. A, Course of ANA positivity in children. B, ANA , p-ANCA, and c-ANCA titers in child #4. C, ANA positivity in child #5; D: Course of anti–cardiolipin IgG titers (no longer detectable after second vaccine dose) in child #6. E, Course of anti-cardiolipin IgM titers in children #7, #8, and #9 after second vaccine dose. F, Course of anti–thyroid microsomal antibody titers for child #10. ANA indicates anti–nuclear antibodies; c-ANCA, cytoplasmic anti-neutrophil cytoplasmic antibodies; Ig, immunoglobulin; MPL, IgM phospholipid units; p-ANCA, perinuclear ANCA; T0, before vaccination; T1, 1 month after first vaccine dose; T2, 1 month after second vaccine dose; T3, month 12.

To the best of our knowledge, there have been no reports to date of the development of autoimmunity following hepatitis A vaccine in children. In our study, 10 of the 40 children studied had various autoantibodies after receiving the 2 vaccine doses. Although none of them had clinical features of autoimmune disease, 2 children continued to be ANA-positive at month 12 of the study.

How vaccines induce autoimmunity is not yet clearly understood. It is thought that one of the mechanisms might be molecular mimicry, whereby, thanks to its structural similarity, the vaccine antigen, or directly, a host antigen, might trigger autoimmunity [1,3]. A second possibility is that a vaccine adjuvant might induce polyclonal stimulation of B cells, thereby increasing autoantibody production [2]. Additionally, increased expression of major histocompatibility complex Class II molecules by nonprofessional antigen-presenting cells (APCs) and activation or dysregulation of T and/or B lymphocytes by vaccines or their adjuvants have been proposed as an explanation for the possible induction of autoimmune diseases by vaccines and other immunostimulating substances [1,2]. While no single factor can be identified as the leading cause of autoimmunity, an interplay of factors is the likeliest hypothesis [14].

Alum is an inorganic reagent used to augment the immunogenicity of vaccines. Examples are aluminium phosphate and aluminium hydroxide, which are the most common adjuvants used in human vaccines. Adjuvants seem to modulate a common set of genes, promote APC recruitment, and mimic specific sets of conserved molecules such as bacteria components, thus increasing the innate and adaptive immune responses to vaccines. Alum has the potential to cause severe local and systemic adverse effects including sterile abscesses, eosinophilia, and myofasciitis; fortunately, most of these effects are relatively rare [6].

It has been suggested that infections can trigger autoimmune diseases; vaccines are involved in these processes and adjuvants have also been implicated in the etiology of these disorders [3]. Vaccines that have been associated with autoimmunity are tetanus, typhoid, hepatitis B, hemophilus influenza B, and influenza vaccines [15-18]. Hepatitis B vaccine has been linked on many occasions to the onset of autoimmune disorders, such as erythema nodosa, vascular inflammation, transverse myelitis, autoimmune thrombocytopenia, Evan's syndrome, rheumatoid arthritis, ankylosing spondylitis, and systemic lupus erythematosus. One case-control study, for example, showed acute central nervous system inflammatory demyelination in 349 children matched with 2941 healthy controls and demonstrated an increased risk of a demyelinating event 3 years after hepatitis B vaccination [19]. A causal relationship has also been identified between the oral polio vaccine and transverse myelitis or type 1 diabetes mellitus and hemophilus influenza type B [17,20]. Finally, several cases of arthritis have also been reported following vaccination [21,22].

Autoantibodies are a hallmark of autoimmune disease, but they can also be positive in apparently healthy individuals. In many studies, an increase in autoantibodies is seen following vaccination, but in most cases, this increase is temporary. A recently published study of a cohort of healthy adults demonstrated that the influenza vaccine was capable of inducing an increase in autoantibody levels [23].

Transiently or persistently increased levels of autoantibodies or the appearance of new autoantibodies has also been demonstrated in up to 15% of apparently healthy adults after the influenza vaccination [24]. Antiphospholipid antibodies are a heterogeneous family of antibodies that react to negatively charged phospholipids or phospholipid–protein complexes. Antiphospholipid syndrome occurs due to the production of antibodies against phospholipid, a cell membrane substance. A study that investigated the induction of these antibodies in 85 healthy volunteers vaccinated with the recombinant DNA hepatitis B vaccine reported a transient increase in aCL titers in 2 participants and a transient increase in anti-b2-GPI titers in 1 participant [25]. In our study, although there was no clinical evidence of autoimmune disease, some children tested positive for autoimmunity markers. After the first vaccine dose, 4 children were ANA-positive and 1 was aCL IgG–positive. After the second dose of vaccine, 2 of the children continued to be ANA-positive and 3 had aCL IgG antibodies. Since there is increasing experimental evidence that antiphospholipid antibodies are induced by a range of infectious agents, we decided to study autoantibodies after checking for the absence of infection. Although 1 child tested ANA-positive until the end of the study, none of the participants developed clinical signs of overt autoimmune disease or demonstrated aPL-related thrombotic events during the 12-month follow-up. Additionally, leukopenia was only observed temporarily in 1 child. A review of the literature did not yield any information regarding the development of leukopenia following vaccinations, although there have been reports of idiopathic thrombocytopenia in association with measles, mumps, and rubella vaccination [26].

With respect to genetic susceptibility to autoimmune diseases, even if individuals are screened prior to vaccination, it is not certain whether or not they will develop an autoimmune disease. The majority of children known to have an autoimmune disease tolerate vaccines well and do not experience exacerbations. Current evidence suggests that the vaccination of healthy individuals seldom induces the production of autoantibodies and when it does, these usually appear transiently and in low titers [25]. However, Knip et al [27] assessed the predictive characteristics of glutamic acid decarboxylase and islet antigen-2 antibodies for type 1 diabetes mellitus over a period of 27 years in a population-based series of 3475 Finnish individuals initially aged 3 to 18 years. They showed that 1-time screening of these antibodies would identify approximately 60% of individuals who would develop type 1 diabetes in 27 years. They showed both positive and inverse seroconversions of these antibodies, reflecting the dynamic process of ß-cell autoimmunity.

We found transient ANA positivity in 25% of children after hepatitis A vaccination; just 2 of these children remained positive but there was no evidence of autoimmune disease. However, other factors such as ethnicity and environment may influence the appearance of autoantibodies following vaccination. In conclusion, a larger study is required to examine the relationship between hepatitis A vaccination and autoimmunity development. Clinical evaluation of potential autoimmune side effects and the establishment of appropriate laboratory test protocols are crucial.

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