From Tolerance to Autoimmunity: Is There a Risk in Early Life Vaccination?

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Summary
The potential for vaccines to act as triggers of autoimmune reactions has received much recent attention. Such an association is very poorly defined mechanistically, but may potentially involve epitope mimicry between vaccinal and self antigen, or the immuno-stimulatory effects of vaccine adjuvant. If such reactions occur, they are more likely to involve adults than infants in early life, as a reflection of the immunological immaturity of the newborn. There has been a recent focus in immunology on the link between innate and adaptive immunity provided by dendritic cells and the range of Toll-like receptors (TLRs) that are the point of first contact of these cells with microbial antigen. These interactions appear to determine the nature of the subsequent adaptive immune response and whether it may be mediated by Th1, Th2, Th17 or T regulatory populations. TLR interactions may also be significant in the induction of vaccinal immunity and agonists of these receptors are being developed as potential vaccine adjuvants. There are differences in cytokine production of adult and newborn dendritic cells, and these differences must be considered in the application of such novel adjuvants to products intended for either age group.

Introduction
In human medicine, wide attention has been given to the proposed associations between autoimmune inflammatory events and vaccination, although the scientific proof for such cause and effect is limited. The immaturity of the newborn immune system is generally considered as a safeguard against the development of this type of reaction following neonatal immunization. However, as vaccine technology progresses the potential for early life triggering of such aberrant immunological events should not be dismissed.

Mechanisms of Vaccine-Associated Autoimmunity
Two main hypotheses have been proposed to explain this immunological association: epitope mimicry and the effect of adjuvant. Epitope mimicry implies that one of the antigens included in a given vaccine may share structural similarities with self-antigens. Consequently, the immune response to this vaccine antigen may subsequently extend to one directed against the host cells that express the self-antigen.

Alternatively, it is now recognized that certain types of adjuvant may drive the differentiation of pathogenic, auto-aggressive lymphocytes (T cells in particular), with the potential of inducing damage to host tissues. The mechanism involved in this evolution from silent autoimmunity to autoimmune disease has been unravelled over the past decade. Adults are equipped with B and T lymphocytes able to recognize self-antigens. In the majority of individuals, this autoimmune potential remains silent. But in some cases, the silenced cells may be turned into aggressive cells causing autoimmune disease when the organism is exposed to certain molecules of microbial origin. This has been clearly demonstrated for autoantigens causing type I autoimmune diabetes. This “activation” takes place.
through signalling, which involves Toll-like receptors (TLRs).

**Toll-Like Receptors and the Innate Response to Microbes**

A set of highly conserved receptor molecules, the TLRs, respond to a variety of microbial molecules, which act as ligands for these receptors. This occurs during the early phase of exposure to microbes, which corresponds to the activation of innate immunity. By setting in motion biochemical cascades involving adapter molecules, kinases and sequences of phosphorylation events, this gives rise to the activation of transcription factors and the production of a large array of proteins, which characterize the inflammatory response (Beutler, 2004).

In humans, ten members of the TLR family have been described so far. Each TLR is specific for a given type of microbial molecule, including glycopeptides (ligand for TLR2), flagellin (ligand for TLR5) and lipopolysaccharide (LPS; ligand for TLR4). Some TLRs have different types of nucleic acids as ligands. For example, double stranded RNA is the ligand for TLR3, single stranded RNA is the ligand for TLR7 and bacterial CpG DNA is the ligand for TLR9 (Takeda and Akira, 2005). These TLRs are expressed on the surface or in the endosomal compartments of antigen-presenting cells (APCs), in particular, dendritic cells (DCs). TLRs are also expressed on a wide variety of other cell types, including neutrophils, mast cells, basophils, eosinophils, epithelial and endothelial cells, and other cells that take part in the adaptive immune response.

**The Role of Toll-Like Receptors in the Induction of T-cell responses**

DCs, the professional APCs to naïve cells of the immune system, receive signals through a wide variety of pattern recognition receptors such as TLRs. Thus in addition to presenting antigen to naïve T cells in an appropriate MHC context, the range of co-stimulatory signals delivered to the T-cell by the APC is determined by TLR ligation.

Molecules of microbial origin, named pathogen associated molecular patterns (PAMPs), engage the TLR and bind to it, thereby eliciting a cellular programme (the expression of accessory molecules and production of a number of cytokines) that leads to the maturation of DCs. Only mature DCs will be able, through appropriate antigen presentation, to stimulate naïve T cells such that they differentiate into effector T cells. The type of effector T-cell that evolves from the naïve cell is greatly influenced by the pattern of cytokines induced by the TLR engagement.

**Autoimmune Diseases and TLR Signalling**

In certain experimental settings, the activation of TLRs results in an autoimmune disease. Experimental autoimmune encephalomyelitis (EAE) is the animal model for the study of multiple sclerosis in which there is auto-reactivity to antigens such as myelin. A selection of recent papers shows that in animals tolerant to myelin antigens, but harbouring T cells able to recognize these antigens, the inoculation of ligands to TLR4 or TLR9 triggers the development of EAE (Waldner et al., 2004). A further study using wild-type mice showed that LPS injection induced relapses of EAE (Nogai et al., 2005). More recently, TLR9 was shown to be important for the induction of EAE in an animal model where, although no TLR9 ligand was injected, endogenous ligands seemed to play a role in the development of the disease (Prinz et al., 2006).
TLR signalling also has a role in the induction of autoimmune diabetes for which there are also excellent experimental rodent models. In one such model where T cells programmed to attack the β cells of pancreatic islets remain physiologically silent, these T cells become activated upon injection of ligands for TLR3 or TLR7 (Lang et al., 2005). This study demonstrated that type I interferons, especially IFN-α, were involved in this process; by inducing Th1 polarization, expression of MHC molecules on pancreatic β cells and activation of islet-infiltrating cytotoxic T cells. The same authors also demonstrated that the progression of autoimmune hepatitis could be accelerated following the injection of TLR3 ligands (Lang et al., 2006).

Systemic autoimmune diseases can also be triggered by TLR engagement. Recently, two back-to-back studies showed that TLR7 (and to a lesser extent, TLR9) present on B cells can, when engaged, precipitate the onset of experimental lupus erythematosus (Christensen et al., 2006; Berland et al., 2006). The reason why TLR9 is less efficient in accelerating this autoimmune process is not known.

**TLR Agonists as Vaccine Adjuvants**

Newer vaccines, some of which are already available, are based on adjuvants that engage some of the previously mentioned TLRs. Monophosphoryl lipid A is now included in several vaccine formulations (e.g., papillomavirus vaccine). This molecule is the ligand for TLR4 on myeloid DCs. Vaccines against cancer and against allergic diseases, currently undergoing clinical trials, contain CpG DNA, a ligand for TLR9 on plasmacytoid DCs. A molecule named Resiquimod (R-848), engages TLR7/8, and has recently been shown to be efficient at inducing tumour necrosis factor (TNF) α and IL-12p40 in cord blood, allowing a degree of circumvention of neonatal APC immaturity (Levy et al., 2006). Both of these adjuvants (CpG and R-848) induce the secretion of IFN-α by plasmacytoid DCs, which may play a role in the induction and/or acceleration of autoimmune diseases.

**Type I Interferon Production by Neonatal Plasmacytoid DCs**

Adult and neonatal plasmacytoid DCs express comparable levels of TLR9 and TLR7. However, when comparing their ability to produce IFN-α in response to CpG oligonucleotides or R-848, an extremely low secretion is observed for neonatal pDCs, both at the protein and mRNA levels (De Wit et al., 2004). This is reassuring in terms of early life immunization; however, the production of IL-27 and IL-12 by myeloid DCs should also be examined in neonates.

**Production of Th1-Driving Factors by Neonatal Myeloid DCs**

The ability of myeloid DCs to produce IFN-β upon TLR4 stimulation (via LPS) differs strongly between adults and newborns. The latter produce scarcely detectable IFN-β protein and mRNA (Aksoy et al., 2007). This suggests that IFN-β will not substitute for IFN-α in terms of Th1 polarization. In terms of other cytokines linked to Th1 polarization, we previously demonstrated that IL-12 synthesis by TLR-stimulated mDCs was strongly limited in neonates (Goriely et al., 2004) and a similar defect was recently observed for IL-27 (unpublished data).

**Production of Other Pro-Inflammatory Cytokines**

In spite of the defects mentioned above, neonatal myeloid DCs are able to produce other pro-inflammatory cytokines upon TLR4 stimulation. They do so in comparable levels to adult DCs, at least for IL-8, TNFα and IL-6 (Goriely et al., 2001). These cytokines depend on the activation of transcription factor NF-kB, which is activated through TLR3 and/or TLR4 engagement. But IFN-β, IL-12 and IL-27 depend on the activation of an alternative transcription factor, IRF3. This was confirmed using IRF3-deficient mice, which proved to be profoundly deficient in IL-12 and IL-27 upon LPS stimulation (Goriely et al., 2006; Molle et al., 2007).

Using micro-array technology, the expression of genes depending upon NF-kB or IRF3 activation was explored in neonatal and adult TLR4-stimulated DCs. No statistically significant difference was observed regarding the genes regulated by NF-kB. In contrast, IRF3-dependent gene expression was generally lower in neonatal compared with adult cells (Aksoy et al., 2007). This has been confirmed by mRNA quantification (real-time quantitative polymerase chain reaction), for instance for CXCL9, CXCL10, CXCL11 and TNFSF10.

There is now further evidence that the defect in IRF3 activation in neonatal DCs lies in the nuclear assembly of IRF3 (translocation takes place) with a co-factor named CBP/p300, impairing DNA binding (Aksoy et al., 2007). The different functioning of neonatal DCs compared with adult DCs is caused by an impaired activation of IRF3-dependent genes. The main differences between adult and neonatal DCs are represented in Fig. 1.

**Th17: Changing the Picture**

A new subset of CD4+ T cells was identified in 2005, now named the Th17 subset (Wynn, 2005). Production of IL-23 by mature DCs plays a major role in the maintenance and amplification of Th17 T cells. These Th17
Effector T cells produce IL-17, a very important pro-inflammatory cytokine, which acts upon a large number of cell types. Evidence is now accumulating which indicates that the most important cytokines for autoimmune reactions are not IL-12 and IFN-γ but IL-23 and IL-17. This paradigm shift has been shown in experimental diabetes, in inflammatory bowel disease and in EAE. It is also known that IL-17 is over-expressed in multiple sclerosis, rheumatoid arthritis and psoriasis (Cua et al., 2003; Weaver et al., 2006; Mensah-Brown et al., 2006).

Initially, the cytokine IL-12 was suggested to be a key factor in mediating autoimmune reactions, but it is now believed that IL-23 is of greater importance. IL-12 and IL-23 are structurally similar. They are both heterodimeric cytokines and share a common p40 chain, differing by only the other sub-unit. All studies that identified IL-12 as a key player in autoimmune responses were based on IL-12 neutralization with monoclonal antibodies which have proven to be directed against p40 and so are able to neutralize IL-23 at the same time. Furthermore, although both cytokines have different receptors, these receptor molecules share a common chain (IL-12Rβ1).

As a result, when TLR4 is engaged by an adjuvant and a DC is activated, this induces the polarization of Th1, Th2, Th17 or Regulatory lymphocytes, depending on the cytokine pattern produced by the DC. As a consequence, a given TLR4 ligand may drive, depending on a number of conditions, different types of T-cell responses (even of opposing types) (Goldman, 2007).

Nevertheless, and of importance for neonatal immunization, IRF3 is not required for the production of IL-23, as opposed to IL-12. Moreover, neonatal DCs not only efficiently produce IL-23 upon TLR4 activation, but they produce significantly higher levels of IL-23 than do adult DCs (Vanden Eijnden et al., 2006). Therefore, there is potential for driving effector pathogenic autoreactive T cells, since neonatal T cells are able to respond to IL-23 (Vanden Eijnden et al., 2005). This has been evidenced for both CD4+ and CD8+ T cells, where polyclonal activation of neonatal CD8+ T cells induces a high production of IL-17. Furthermore, IL-27 was very recently shown to block the differentiation of Th17 cells. This cytokine appears to have a dual role, since it also favours Th1 differentiation (Colgan and Rothman, 2006). Indeed, IL-27 regulates autoimmune neuro-inflammation by repressing pathogenic Th17 cells, as observed in two different models (Batten et al., 2006; Stumhofer et al., 2006). Since production of IL-27 is limited in early life, newborns might lack an important regulatory mechanism involved in the differentiation of Th17 cells.

The potential relationship between TLR-based vaccines and autoimmunity remains an open question that needs a careful risk assessment. This risk probably exists at a low level. It may be a responsible approach to acknowledge and evaluate it, in order to avoid allegations that, afterwards, are difficult to contravene. There might also be a need for specific early life considerations. For adjuvants triggering TLR7, 8, or 9, the risk may be limited by the decreased type I interferon synthesis. Vaccines targeting TLR4 might require a more cautious approach since they would be expected to induce high IL-23 and low IL-27 synthesis.

References


