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Minireview

Adjuvant activity of type I interferons

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Abstract

Type I interferons (IFNs) produced primarily by plasmacytoid dendritic cells (pDCs) as part of the innate immune response to infectious agents induce the maturation of myeloid DCs and enhance antigen presentation. Type I IFNs also enhance apoptosis of virus-infected cells, stimulate cross priming and enhanced presentation of viral peptides. Type I IFNs are powerful polyclonal B-cell activators that induce a strong primary humoral immune response characterized by isotype switching and protection against virus challenge. Type I IFNs stimulate an IgG2a antibody response characteristic of Th1 immunity when ad-mixed with influenza virus vaccine and injected intramuscurarly (i.m.) or administered intranasally. The adjuvant activity of type I IFNs has been shown to involve direct effects of IFN on B-cells, effects on T-cells, as well as effects on antigen presentation. Oromucosal administration of type I IFNs concomitantly with i.m. injection of vaccine alone can also enhance the antibody response to influenza vaccination by enhancing trafficking of antigen-presenting cells towards the site of vaccination. Recombinant IFNs are potent adjuvants that may find application in both parenterally and mucosally administered vaccines.

Keywords: adjuvant; apoptosis; cytokines; interferons; Toll-like receptor (TLR); vaccination.

Introduction

Vaccination reduces the morbidity and mortality associated with numerous bacterial and viral infections in at risk groups, including the aged and individuals, with an impaired immune response, but is not totally protective in all recipients (Oxford et al., 2003). For example, the protection afforded by the commonly used influenza subunit vaccines is thought to be due principally to the production of antibodies to viral hemagglutinin and the hemagglutination inhibitory (HI) antibody titer is used as a surrogate marker of protection. It is generally accepted that an HI antibody titer of 1:40 or greater can confer protection. This is attained, however, in only 50% of elderly subjects (Oxford et al., 2003). Thus, there is an unmet need for an effective non-toxic adjuvant capable of enhancing the antibody response to influenza and other vaccines. Cytokines, including the type I interferons (IFN α and IFN β), play an important role in B-cell activation and the regulation of the humoral response (Le Bon et al., 2001, 2006a). Cytokines produced by recombinant DNA technology may therefore constitute a novel group of candidate adjuvants (Lien and Golenbock, 2003).

Activation of the type I interferon pathway

Key populations of cells, including dendritic cells (DCs), distributed throughout the peripheral tissues act as sentinels capable of recognizing infectious agents through pattern-recognition receptors (PRR). These include the Toll-like receptor (TLR) family of cell surface and endosomal membrane receptors (Uematsu and Akira, 2007) and the retinoic acid-inducible gene I (RIG-I)-like cytosoloic receptor proteins RIG-I, MDA5 and LGP2 (Yoneyama and Fujita, 2007). A total of 13 members of the TLR family have been identified in mammals (Uematsu and Akira, 2007). Each TLR mediates a distinctive response in association with different combinations of four Toll/IL-1 receptor (TIR) domain-containing adaptor proteins (MyD88, TRIF, TIRAP/MAL and TRAM). All the TLRs except TLR3 interact with MyD88. TLR3, which recognizes single-stranded or double-stranded viral RNA, is localized in the endosomes of myeloid DCs and requires acidification of vesicles for activation. TLR3 signals via TRIF and activates TBK1/IKKε which phosphorylates the interferon regulatory factor 3 (IRF3) and NF-κB, resulting in production of IFN β (Hemmi et al., 2004; Perry et al., 2004). The RIG-I-like receptor proteins are DExD/H box RNA helicases two of which, RIG-I and MDA5, carry caspase activation and recruitment domain (CARD)-like motifs at the N-terminus (Yoneyama and Fujita, 2007). The CARD domain interacts with IPS-1 resulting in activation of IRF3 and NF-κB and production of IFN β. Thus, activation of PRRs leads to the production of pro-inflammatory cytokines, including type I IFNs, and activation of the innate immune response.

DCs signal principally through TLRs, while RIG-I-like receptors predominate in other cell types. Two major DC sub-sets can be distinguished in man, $CD11c^+$ monocyte derived myeloid DCs, present in most tissues, and $CD11c^-$ plasmacytoid DCs (pDCs), present principally in lymph nodes. pDCs are the principal producers of type I IFNs in response to viruses (Steinman and Hemmi, 2006). pDCs express high levels of TLR7/8 and TLR9 that recognize single-stranded RNA (ssRNA) and CpG DNA, respectively (Hemmi et al., 2000; Diebold et al., 2003; Heil et al., 2003). Activation of both TLR7/8 and TLR9

leads to the formation of a complex with MyD88 and phosphorylation of IRF7 and production of high levels of type I IFNs (Uematsu and Akira, 2007).

In man, the type I IFN gene family is located on the short arm of chromosome 9 and encodes 12 functional IFN α, and single IFN β, IFN ω, IFN ε and IFN κ subtypes. All type I IFNs bind to a common high-affinity cell surface receptor composed of two trans-membrane polypeptides, IFNAR1 and IFNAR2. IFN binding results in the phosphorylation and activation of a transcription complex, ISGF3, composed of the signal transducer and activator of transcription (STAT1), STAT2 and IFN regulatory factor 9 (IRF9). Translocation of this complex to the nucleus results in the transcriptional activation of a specific set of genes that encode the effector molecules responsible for mediating the biological activities of the type I IFNs. All type I IFNs induce comparable qualitative biological activities although quantitative differences in the activity of different subtypes has been reported (Masci et al., 2006).

Effect of type I interferons on the innate immune response and induction of adaptive immunity

Type I IFNs stimulate DC maturation by increasing expression of co-stimulatory molecules, including CD40, CD80 and CD86, and major histocompatability complex (MHC) antigens (Steinman and Hemmi, 2006). Upon activation, DCs migrate from the peripheral tissues to the secondary lymphoid organs where they present pathogen-derived antigens to naive T-cells leading to the adaptive antigen-specific immune response. Trafficking of DCs is also enhanced by type I IFNs which increase expression of several chemokines and chemokine receptors (de Veer et al., 2001). It has been shown that type I IFNs play an important role in cross-priming (Beignon et al., 2003; Le Bon et al., 2003, 2006b). Namely, following activation, DCs present foreign virus-derived peptides on MHC class I antigens to $CD8^+$ T leading to $CD8^+$ T-cell activation (Le Bon et al., 2003). IFN stimulated cross-priming appears to be independent of the CD40 ligand-CD40 interaction between DCs and CD^+ T-cells (Beignon et al., 2003; Le Bon et al., 2003, 2006b). Thus, type I IFNs produced primarily by pDCs play a key role in the innate immune response to virus infection and in the induction of the primary adaptive-immune response. Type I IFNs are also powerful polyclonal B-cell activators which induce a strong primary humoral immune response characterized by isotype switching and protection against virus challenge (Le Bon et al., 2001). Thus, type I IFNs have been shown to induce B-lymphocytes to differentiate into antibody producing plasma cells and to be necessary for the production of both specific and polyclonal IgGs in response to influenza infection (Jego et al., 2003). Furthermore, type I IFNs have been shown to enhance the primary antibody response to a soluble antigen *in vivo* and to enhance the production of all IgG sub-classes (Le Bon et al., 2001, 2006a,b). Type I IFNs also enhance long-term antibody production and immunological memory (Le Bon et al., 2001, 2006a). Thus, not surprisingly type I IFNs have been shown to be powerful adjuvants when ad-mixed with influenza vaccine and injected intramuscularly (i.m.; Proitti et al., 2002). Type I IFNs also play a key role in adjuvant-induced T-helper type I (Th1) responses, and induction of type I IFNs is required for the activity of well-known adjuvants, such as Freund's adjuvant and certain CpG oligonucleotides (Le Bon et al., 2001). The adjuvant activity of type I IFNs has been shown, using mice deficient in the type I IFN receptor on specific cell populations, to involve both direct effects of IFN on B-cells, as well as effects on T-cells, most probably $CD4^+$ T-cells (Le Bon et al., 2003, 2006a,b). Production of B-cell stimulatory factors, including BAFF and APRIL, by IFN-stimulated DC may also play a role in the adjuvant activity of the type I IFNs (Litinskiy et al., 2002). Although the precise mechanism(s) of the IFN stimulation of the humoral response remains to be elucidated, possible mechanisms include enhanced antigen-triggered proliferation and differentiation of B-cells, and protection of both B-cell and helper T-cells from apoptosis leading to enhanced and prolonged antibody production (Le Bon et al., 2006a). Both recombinant IFN α 2 and mixtures of naturally occurring IFN α subtypes and IFN β have been reported to exhibit adjuvant activity (Proitti et al., 2002; Tovey et al., 2006). Although the relative adjuvant activity of individual type I IFNs remains to be determined, it will be of interest to determine the adjuvant activity of IFN α 1, which is the major IFN α subtype produced by pDCs in response to virus infection (Izaguirre et al., 2003).

Type I IFNs exert both direct and indirect effects on T-cells and play a key role in the fine-tuning of their activity, by delivering both stimulatory and inhibitory signals. In this manner, type I IFNs contribute to the homeostasis of the immune response. Thus, treatment of naive $CD4^+$ T-cells with type I IFNs delays their entry into the cell cycle after T-cell receptor (TCR) triggering, while IFN treatment of activated T-cells does not affect cell proliferation (Dondi et al., 2003). Indirect effects of the type I IFNs on T-cell activation include induction of co-stimulatory molecules, CD80 and CD86, on antigen presenting cells (APCs) which in the presence of antigen stimulate T-cell activation and prevent induction of anergy (Marrack et al., 1999). Type I IFNs also act directly on memory T-cells to increase their survival and indirectly contribute to the survival of memory T-cells through stimulation of IL-15 production by APCs (Zhang et al., 1998; Marrack et al., 1999). Virus stimulated pDCs produce large amounts of IFN α and prime naive $CD4^+$ T-cells to differentiate into IFN γ/IL-10 producing cells possessing anergic and regulatory properties (Levings et al., 2001; Kawamura et al., 2006). Induction of this functional phenotype is dependent on the presence of IFN α. Type I IFNs also attenuate the generation of antigen-specific $CD8^+$ T-cells through the induction of $CD4^+$ Tr1 cells (Dikopoulos et al., 2005). Thus, type I IFNs may play an important role in suppressing an excessive inflammatory response while at the same time stimulating the humoral response.

Effect of type I interferons on apoptosis and antigen presentation

Human DCs phagocytose virus-infected apoptotic cells and present peptide epitopes on MHC class I molecules

resulting in activation of viral antigen-specific $CD8^+$ T-cells, a process known as cross-priming (Blachère et al., 2005). Type I IFNs can modulate this process in two ways. Firstly, type I IFNs can activate the transcription of genes involved in antigen-processing, such as TPA1 (transporter associated with antigen processing; Epperson et al., 1992), LMP2 (low molecular mass polypeptide 2) or LMP7 (Der et al., 1998), resulting in enhanced antigen presentation. Secondly, type I IFNs can activate a number of components involved in the apoptotic pathway, and thereby stimulate apoptosis. Although type I IFNs have been reported to trigger apoptosis in tumor cells (reviewed in Pokrovskaja et al., 2005), in untransformed cells type I IFNs appear to potentiate rather than induce apoptosis. Thus, type I IFNs activate the transcription of a number of genes which encode pro-apoptotic proteins, including pro-caspase 2 and 8, FAS, FAS ligand and the transcription factors IRF1 and IRF3 (de Veer et al., 2001). IRF1 and IRF3 play a central role in the regulation of the IFN system. They are the principal transcriptional activators of the type I IFNs and are themselves in turn induced by type I IFNs constituting a positive feedback loop. Furthermore, IRF1 and IRF3 are also involved in the pro-apoptotic activity of the type I IFNs. Thus, the presence of infectious virus, inactivated viral particles or viral components, such as dsRNA, induce post-translational modification (e.g., phosphorylation) of IRF1 and IRF3 resulting in marked transcriptional activation of the apoptotic gene NOXA resulting in apoptosis of virus-infected cells (Lallemand et al., 2007), increased numbers of apoptotic vesicles and enhanced presentation of viral peptides by IFN-activated DCs. Induction of CD69 by both type I IFN and IFN γ (Sun et al., 1998) has also been reported to stimulate apoptosis by a mechanism that remains unclear (Walsh et al., 1996). Together, these observations suggest that type I IFNs can stimulate antigen presentation both by inducing the maturation of myeloid DCs and enhancing the presentation of viral peptides, as well as by enhancing apoptosis resulting in increased numbers of apoptotic vesicles, that can be processed directly by DCs.

Type I interferons and mucosal immunity

Nasal immunization with inactivated whole virus vaccines or virus-like particles induces secretory IgA and systemic IgG antibody responses that afford protection from respiratory infections and prevent dissemination of the infection to extra pulmonary sites (Reuman et al., 1990; Cartner et al., 1998). Mucosal administration of soluble proteins in general does not, however, elicit a strong antibody response (Akbari et al., 2001; Jones et al., 2001). Type I IFNs ad-mixed with the influenza subunit vaccine and administered intranasally have been shown to afford complete protection of animals against virus challenge, while vaccine alone was only partially effective (Bracci et al., 2005). Type I IFNs are produced at mucosal surfaces as part of the innate immune response to infectious agents. Oromucosal (o.m.) administration of recombinant IFN α mimics o.m. production of IFN and has been shown to confer protection against virus infection and tumor cell multiplication (Tovey and Maury, 1999). Protection occurs through stimulation of cellular immunity in the absence of circulating levels of IFN (Eid et al., 1999). The absence of detectable circulating levels of IFN α following o.m. administration may well account for the absence of the systemic toxicity, including myelosuppression and liver toxicity, associated with parenteral administration of similar doses of IFN α (Tannir et al., 2006; Sirohi et al., 2007). In particular, o.m. administration of IFN α stimulates both the maturation of DCs and antigen presentation and the $CD4^+$ Th1 lymphocyte response to foreign antigens (Tovey and Maury, 1999; Tovey et al., 2006).

Treatment of C57Bl/6 mice with recombinant IFN α was found to markedly enhance the humoral response to a commercially available influenza vaccine (Vaxigrip™) when ad-mixed with the vaccine and injected i.m. (Tovey et al., 2006). In agreement with a previous report (Proitti et al., 2002), IFN α treatment was found to markedly increase the serum levels of all four classes of influenza-specific immunoglobulins (IgG, IgG1, IgG2a, and IgA) tested. The effect of IFN α on influenza-specific Ig production was dose-dependent with the greatest increase in Ig sub-classes observed at a dose of 10^5 IU, the highest dose of IFN tested. Similarly, o.m. administration of the same dose of IFN α concomitantly with i.m. injection of vaccine alone was found to enhance the antibody response to influenza vaccination. The use of transgenic mice expressing an EGFP reporter gene regulated by an IFN responsive promoter has shown that IFN activated (green) cells were present in the peripheral circulation of influenza vaccinated mice as early as 4 h after initiation of i.m. or o.m. IFN treatment and that the principal cell populations activated by IFN treatment included both myeloid DCs and pDCs. These results suggest that the increased influenza-specific Ig response to vaccination observed under these conditions may be related to trafficking of IFN-activated antigen-presenting cells to the site of influenza vaccination. Differential display analysis showed that numerous IFN responsive genes were induced in the spleen and peripheral blood leukocytes of these IFN-treated animals, including Stat1, Mx and the chemokine Crg2, which regulates lymphocyte trafficking (Tovey et al., 2006). Thus, trafficking of IFN-activated antigen presenting cells to the site of vaccination may explain, at least in part, the mechanisms underlying the adjuvant activity of IFN α.

Conclusions

Type I IFNs produced primarily by pDCs as part of the innate immune response to infectious agents play an important role in the initiation of the adaptive antigen-specific immune response and the induction of long lasting virus-specific immunity. Type I IFNs exert their effects both by transcriptional activation of a number of genes, some of which are subject to autocrine regulation, and by the induction of other immunoregulatory and inflammatory cytokines which potentiate the immune stimulatory effects of the type I IFNs. Recombinant IFNs ad-mixed with influenza vaccine and injected i.m. or administered intranasally have been shown to be potent adjuvants which enhance protection against virus challenge. Thus, recombinant IFNs hold considerable promise as a new class of adjuvants. Parenteral administration

of recombinant IFNs is associated, however, with significant toxicity, including a flu-like syndrome (Tannir et al., 2006; Sirohi et al., 2007). More serious myelosuppression and neuropsychiatric effects also appear after long-term treatment (Tannir et al., 2006; Sirohi et al., 2007). Although adjuvants are defined as 'substances which increase the immune response to an antigen with which they are mixed' (Lien and Golenbock, 2003), o.m. administration of IFN α has been reported to enhance the Ig response to vaccination. Thus, o.m. administration of type I IFNs concomitantly with parenteral vaccination may offer an effective well tolerated means of enhancing the efficacy of existing vaccines without the need to reformulate each vaccine as would be the case for a novel adjuvant mixed with a vaccine.

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