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Cytokine therapy for combating animal and human diseases – A review

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Abstract

Disease control in food production animals is normally achieved through the use of antimicrobials, drugs and vaccines. However, the injudicious and wide use of antibiotics and other drugs/chemicals in animal husbandry practices and livestock raising systems have resulted in environmental, food safety and human health concerns, principally due to the emergence of drug-resistant microbes and residual toxicity in foods of animal origin. During recent years, therapeutic interventions with biologics and newer treatment modalities like phages, apoptins, cytokines, avian egg yolk antibodies, stem cell therapy, herbal remedies and others are gaining more and more focus into medical and veterinary sciences. Of these, cytokines therapy is of immense importance and possesses great potential and values for treating human as well as animal diseases. Cytokines, the natural mediators of immunity, include interleukins (IL), interferons (IFN), tumor necrosis factor (TNF), chemokines, adipokines and mesenchymal growth factors. Cytokines have characteristic multiple biological properties (pleiotropism), and affect various stages of embryonic development, disease pathogenesis, and immune responses including of both non-specific and specific ones. Their mode of action on the cells may be autocrine, paracrine or in endocrine manner; mediated by means of various cytokine receptors. Cytokines, their receptors and signal transduction pathways, are promising candidates for treatment purposes and therapeutic interferences due to their multiple functions, regulatory and effector cellular functions, in many diseases. In this regard TNF-TNF receptor interaction and neutralization of any such interaction requires special mention. Cytokines, being good immunomodulators, play crucial role in a vast array of infectious diseases, wherein the use of recombinant cytokines is worth mentioning. The therapeutic applicability of cytokines and modulation of their actions is being explored for a many infectious diseases, autoimmune diseases, immunocompromised patients (acquired immunodeficiency syndrome, AIDS), and in neoplasia. Cytokines possess potential capability in controlling various diseases of animals too and present possible alternatives to traditional / conventional therapeutics. Limitations of cytokine therapy like short half life and adverse affects are also being tackled with innovative novel strategies so as to widen the potent application of these wonderful molecules. The present review discusses the role of cytokines in various therapeutic interventions, anti-tumor effects, and their applications as adjuvants for new generation vaccines both in the field of human as well as animal science / medicine.

Keywords: Cytokines; interleukins; tumor necrosis factor; disease; therapy; adjuvants; anti-tumor activity.

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Introduction

Cytokines are small, less than 30 kDa molecular mass, regulatory glycoproteins secreted by various cells of the immune system (innate as well as adaptive) that help to regulate and boost immunity (Tizard, 2004; Dinarello, 2007). These are the soluble messengers that immune cells produce to either attack an invading microorganism or to talk/communicate to other types of immune cells. Cytokines are either secreted or membrane-bound in nature, and act as intracellular signaling mediators in order to regulate immune system homeostasis. Cytokines bind to the surface membranes of target cells via specific receptors and induce a cascade of reactions, triggering signal-transduction pathways that ultimately alter gene expression in target effects that are involved in innate and adaptive immunity (Randall and Goodbourn, 2008). Cytokine-receptor binding exhibit very high affinity and therefore even at very low concentrations cytokines can mediate profound biological effects. Cytokines regulate the intensity and duration of the immune responses by stimulating or inhibiting proliferation, differentiation, trafficking or emigration of lymphocytes. They exhibit the characteristic of pleiotropy, redundancy, synergy, antagonism, and cascade induction, which altogether help regulate cellular functions in a coordinated, interactive and controlled manner (Tizard, 2004). Cytokines are involved in a broad array of biological activities viz., development and regulation of cellular and humoral immune responses, innate and adaptive immunity, inflammatory response, hematopoiesis, control of cellular proliferation and differentiation. Besides, these have roles in tissue regeneration and healing of wounds (Jan and Feldmann 2004; Cutler and Brombacher, 2005; Dart et al., 2005). The efficacy of cytokine therapy has been demonstrated in several human and animal studies. The availability of recombinant cytokines and soluble cytokine receptors offers the prospect of specific clinical therapies to modulate an immune response (Cutler and Brombacher, 2005; Chabalgoity et al., 2007; Kunz and Ibrahim, 2009). A few cytokines notably interferons and colony stimulating factors, such as GM-CSF, have proven to be therapeutically useful (Varona and Villamayor, 2007; Dhama et al., 2012; Dhama et al., 2013a&b).

Various factors affect their effector functions *viz.*, local concentration, secretory pattern expression along with integrated multi-signaling pathways. The higher frequency of spontaneous cancers in mice that are deficient genetically in type I as well as type II interferon (IFN) receptors or elements that are involved in IFN receptor signaling transduction downstream demonstrate the role of cytokines in immuno-surveillance (Kaplan et al., 1998; Shankaran et al., 2001; Picaud et al., 2002; Dhama et al., 2013b; Kaminskas et al., 2013).

Nowadays due to increasing emergence of drug resistance on behalf of evolving resistant microbial pathogens and injudicious use of antimicrobials especially the antibiotics, residual toxic effects of drugs in food, and emerging and re-emerging pathogenic strains, many novel and safer therapeutic modalities are being explored viz., bacteriophages, virophages, mycophage, apoptins, cytokines, monoclonal antibodies, avian egg yolk antibodies, gene therapy, siRNA and gene silencing, stem cell therapy, nanomedicines, nutritional immunomdulation, panchgavya and herbal remedies (Natesan et al., 2006; Dhama et al., 2005a&b; Dhama et al., 2008a&b; Chakravarthi and Balaji, 2010; Shirley et al., 2011; Mahima et al., 2012, 2013ab&c; Deb et al., 2013; Dhama et al., 2013ab&c; Tiwari et al., 2013a&b). The present review discusses the potential role of cytokines in treating various infectious and non-infectious diseases, tumors/cancers and other maladies along with their potent use as vaccine adjuvants for safeguarding health of animals as well as humans.

Cytokines as disease therapeutics

Cytokine research has introduced new therapies that have revolutionized the concept of treatment of diseases. The therapeutic strategy envisages the administration of purified or recombinant cytokines, or the administration of drugs that inhibit the harmful effects of over produced endogenous cytokines (Lowenthal et al., 1999; Jan and Feldmann 2004). Some of the successful cytokine-based therapeutics includes hematopoietic growth factors, colony-stimulating factors and interferons (IFN) for stimulation and progression of various processes; and cytokine antagonists having profound effects on the treatment of inflammatory disorders like inhibitors of tumor necrosis factor (TNF) (Varona and Villamayor, 2007; Dhama et al., 2008b, 2013b; Dhama et al., 2012). Interferons have been identified as efficacious therapeutic agents for treatment of several clinically important diseases in cattle and horses (Moore, 1996; Jan and Feldmann 2004; Dhama et al., 2008b). In some instances, the therapeutic goal of IFN administration is prevention or clinical cure of acute viral infections in animals, or they may serve as adjunctive treatment to lessen clinical manifestations of disease and improve the quality of life. Further, IFNs have the ability to respond to intracellularly bacterial pathogens affecting the host (Kaufmann, 1993). Also, the IFN system evinces extremely powerful antiviral response, which is capable of controlling most of the virus infections in the absence of adaptive immunity (Masihi, 2006; Randall and Goodbourn, 2008).

Cytokines, as adjunctive immunomodulators, play essential role in a vast array of infectious diseases (Hafler, 2007); use of recombinant α-interferons and nucleoside analogs for hepatitis B virus (HBV); pegylated interferons and ribavirin for hepatitis C virus (HCV) (Forton and Karayiannis, 2006) being the examples. They are attributed to the induction of Th_1 immunity (Trapero-Marugan et al*.,* 2006); cryptococcal meningitis associated with HIV (Antachopoulos and Roilides, 2005) and Crohn's disease (Pizarro and Cominell, 2007). Superior therapeutic approach is provided by the immunoglobulin Fc fragment based cytokines (Jazayeri and Carroll, 2008). To ensure an appropriate immune response, vaccine development in every aspect necessitates new types of adjuvant development (Nicholls et al., 2010). In this regard use of cytokines *viz.,* IL-7, 12 and 15 as mucosal vaccine adjuvants are quiete noteworthy (Stevceva et al., 2006); others include granulocyte-macrophage colony-stimulating factor (GM-CSF) along with monocyte chemotactic proteins (MCPs) and macrophage inflammatory proteins (MIPs). They cause increase in recruitment of dendritic cells that are blood-borne along with monocytes to reach interstitial sites where vaccines are delivered (Klavinskis et al., 2010).

It has been suggested that the pharmaco-dynamic potency of a cytokine-based therapeutic agents can be attributed primarily to three factors: cytokine/receptor binding affinity; cytokine/receptor endocytic trafficking dynamics, and cytokine/receptor signaling (Randall and Goodbourn, 2008). It has been also demonstrated that point mutations as well as a polyethylene glycol (PEG) conjugation can be used to increase the potency of therapeutic cytokines (Yoshida et al., 2005; Kaminskas et al., 2013). Currently, several recombinant cytokines, including interferons, colony-stimulating factors and chemokines, are being widely used in clinical practice in humans, especially in treatment of AIDS and hepatitis B. Also, TGF-β, IL-6, IL-10, GM-CSF and soluble cytokine-specific receptors have been reported to treat patients infected with *Mycobacterium avium*; and IL-2 and TNF-α have been used in protection against general mycobacterial infections (Varona and Villamayor, 2007). Analyzing the role of IFN-γ producing T_h1 has given ample evidences regarding their role for reducing growth of *Mycobacterium* and for maintenance of a mononuclear inflammatory response (Bermudez and Kaplan 1995). IL-2 and IFN-γ have been found to enhance the activity of T lymphocytes in eliminating the coccidian parasite (Eimeria) in birds (Lillehoj et al., 2005; Shah et al., 2011). Use of such cytokines along with the newer generation vaccines also holds promise in controlling experimental coccidiosis in birds. The significance of IFN-γ and TNFα in treating mastitis in bovines has also been reported (Fonseca et al., 2009). Both traditionally studied cytokines such as: TNF-α and INF-γ; IL-1, IL-6, IL-4, IL-5, IL10; and TGF-β as well as others recently characterized viz., IL-13, IL-12, IL-18, IL-23 are found to be effective in inflammatory bowel disease (both ulcerative colitis as well as Crohn's disease) (Sanchez-Munoz et al., 2008). In case of other inflammatory diseases like psoriasis and multiple sclerosis IL-12 family of cytokines such as: IL-12 and IL-23 are found to be important mediators and their direct blockade is a key to treat such diseases (Soldan et al., 2004; Barrie and Plevy, 2005).

In order to have proper immune system activity, regulation of TNF ligand and receptor interactions and functions in an appropriate manner is crucial. With the development of an array of autoimmune as well as inflammatory diseases excessive production of various TNF has been given priority by many scientists. Responses of immune cells viz., T cells, APCs, NK cells and NKT cells should be mainly aimed in this regard along with maintenance of Treg cell function thereby facilitating long-term control of disease (Croft, 2009). Analysis of the activity of neutralizing antibodies during preclinical studies that target specifically TNF ligand proteins or of Fc fusion proteins with a TNFR binding to the ligand and thereby blocking the endogenous interaction is a specific approach. Assessment of the effect of blocking the TNF ligand-receptor interactions have been done in models of inflammatory diseases. These include: allergy and bronchial asthma; organ transplantation and graft-versus-host disease (GvHD) as well as atherosclerosis; autoimmune disease including experimental autoimmune encephalomyelitis (EAE), diabetes, colitis, adjuvant or collagen induced arthritis and systemic lupus erythematosus (Taylor et al., 2002). Neutralization of any one of the TNF-TNFR interactions ultimately results in overpowering of conditions. In most cases, it is precisely linked to decline in CD4+ or CD8+ T cell activity or results in the impairment of NK- and NKT-cell function in the other way (Zimmerer et al., 2012).

Besides the treatment of infectious diseases, for certain non-infectious diseases also the importance and role of cytokines has been identified. Erythropoietin has been applied for the treatment of anemia, and TNF-α-antagonists found useful in the treatment of rheumatoid arthritis, which enables the replacement of steroidal anti-inflammatory drugs (Marchesoni et al., 2009; Kotecha et al., 2011). Several cytokines have been reported to be useful for the treatment of inflammatory dermatological diseases; the best option is the use of interferons (Asadullah et al., 2002). Different forms of interstitial pneumonia can be treated by administering an IL-13 immunotoxin chimeric molecule (Park et al., 2009). Allergic encephalomyelitis can be inhibited by a single injection of therapeutic cytokines like IL-4, IFN-

β and transforming growth factor (TGF-β), as DNA-cationic liposome complex directly into the central nervous system (Croxford et al., 1998). Also, neutropenia, which is commonly observed during numerous pathological conditions or with cancer chemotherapy, can be reversed by administering colony stimulating factors (CSFs).

The contribution of cytokines in the pathogenesis of rheumatic disease when understood have helped in developing novel and creative therapeutic approaches which were not available previously. Clinical trials are already available in case of rheumatoid arthritis (RA). Therapeutic approaches for the purpose of blocking the effects of TNF-α and IL-6 receptor (IL-6R) action are found to be effective in case of many patients in this regard. Small molecules like glucocorticoids have succeeded in treating RA as they can block the transcriptional activities of IL-1 as well as IL-6 and TNF. However, serious adverse effects on immune surveillance due to suppression of the physiologic concentration of certain cytokines can not be overruled and thereby requires precautionary measures (van de Loo et al., 1995; Cohen et al., 2009; Genovese, 2009; Fleischmann, 2012). Low level of IL-4 along with high level of TGF-β; IL-18 and recombinant IL-11 therapy are found to be effective for the treatment of various kinds of arthritis in humans including rheumatoid arthritis (Miossec et al., 1990; Moreland et al., 2001; Dinarello, 2004).

Cytokines play their part in assisting the migration of hematopoietic stem cells and have been found to help in wound healing and tissue regeneration. Cytokines have been used to alleviate trauma during burn injuries, increase the speed of fracture healing and osteogenesis, render positive effects on musculo-skeletal system, play role in pain alleviation and prevention of the rejection of transplanted organs (Dart et al., 2005). Inflammatory cytokines like TNF-α, IL-1, IL-6 and IL-8 mediate and modulate wound healing processes but, if over-expressed, may exacerbate the severity of inflammation. To counter this, cytokines like IL-6, which induces protective acute phase response and IL-4, which turns off or suppresses the inflammatory response are favorable options. Manipulating growth factor profile or wound environment through topical application of cytokine-based therapeutic agents can positively influence the rate and quality of wound repair in animals (Jan and Feldmann, 2004; Dart et al., 2005). TGF-β and activated macrophage supernatant, rich in cytokines, are effective mediators that may facilitate rapid wound healing (Amento and Beck, 1991; O'Kane and Ferguson, 1997).

Nowadays, cytokines like colony stimulationg factors are used in pet animals like dogs and cats for countering multiple granulocyte disorders that include neutropenia, caused by bacterial septicemia, endotoxemia and viral infections, and bone marrow stromal disorders or leukemia (Bravo et al., 1996; Freeman et al., 1997; Yamamoto et al., 2011). Cytokine and anticytokine treatments represent promising approaches for therapy of immune-mediated diseases. Exogenous avian cytokines have also been explored quiet vividly due to recent advances in the field of avian genetics as well as immunology (Rahman and Eo, 2012).

Role of cytokines in tumor therapy

The ability of cytokines to directly inhibit tumor cell growth or to enhance the immune response against tumor cells led different groups to test these agents as anti-tumor molecules (Mocellin et al., 2001; Jan and Feldmann, 2004). Due to their anti-tumor activity, some cytokines are being exploited for therapy of various malignancies. Cytokines such as interferons and interleukins are administered for cancer because of their broad-based immunostimulatory effects including generation of tumor-reactive lymphocytes (Mocellin et al., 2001). IL-2, or aldesleukin, which is indicated for the treatment of adults with metastatic renal cell carcinoma and melanoma, is the most studied cytokine. IL-2 activates cellular immunity in dose-dependent manner and causes in *vivo* release of other immune-boosting cytokines. Immunocytokines show promise during cancer therapy and help improve condition of cancer patients; these have no noticeable risk of cross-resistance with conventional therapeutics (chemo-therapeutics, radiotherapy, and surgery) and therefore, combinational therapy should be exploited (Lode et al., 1998; Lee and Margolin, 2011). IL-2 was the first immunological agent which demonstrated an anti-tumor effect by activating immune effector cells. The most significant results were obtained both in melanoma and renal cell carcinoma and to a lesser degree in hematological malignancies (McIntyre et al., 1992; Bouwhuis et al., 2009). The use of polyethylene glycol modified IL-2 (PEG-IL-2), that retains the *in vitro* and *in vivo* activity of IL-2 but exhibits a markedly prolonged circulating half life, resulted in a significant decrease of IL-2 toxicity in clinical trial (Yang et al., 1995). The association of IFN-α and IL-2 improved the response rate as compared to each cytokine administered alone but its impact on the overall survival remains to be established. IFN-α has a well-known antitumor activity in mouse and human malignant neoplasms; it stimulates proliferation and increases the cytotoxic killing activity of NK cells (Mocellin et al., 2001; Jan and Feldmann 2004). The utility of IFN-α stimulation-based NK cell-mediated cytotoxicity of tumors has been documented while observing the clinical remission of chronic myelogenous leukemia. Similarly, IFN-γ is critical for the efficient upregulation

of the major histocompatibility complex (MHC) pathways and the recognition by tumor-specific CD8 T cells (Muller et al., 1994; Rochman et al., 2009). They induce angiostatic chemokines that inhibit angiogenesis within the developing tumor. Likewise, IL-12 has also a potent antitumor activity, when studied in experimental mouse tumor models (Andrews et al., 2000; Dabrowska et al., 2000). However, the IL-12 activity depends on tumor immunogenicity, cytokine dose, route of injection, production of extracellular matrix proteins and induction of IFN-γ. IL-12 has been reported to work well against Kaposi's sarcoma by downregulating a constitutively active G protein coupled receptor that is encoded by Kaposi's sarcoma-associated herpesvirus, besides inducing the production of IFN-γ (Yarchoan et al., 2007).

For cancer therapy, using recombinant cytokines or cytokine genes is considered as one of the strategy in order to modulate immune responses. In order to control the blood concentration as well as the biological activity, recombinant cytokines have added advantages. Cytokines facilitating type 1 helper T (Th1) cell-mediated responses but not the reactions mediated by Th2 cells are effective for antitumor responses when produced in tumors (Tagawa, 2000). IL12 has exhibited antitumor activity in a variety of murine cancer models including renal cancer, B16 melanoma, reticulum cell sarcoma and C26 colon carcinoma. IL 12, administered in combination with IL-2, induced a rapid and complete regression of primary and metastatic renal tumors and displayed greater antitumor activity than that observed with either IL12 or IL-2 alone. Further, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a membrane-bound cytokine molecule has been shown to be potent apoptosis inducers in a wide variety of cancer cells *in vitro* and has limited tumor growth efficiently *in vivo* conditions also, without damaging normal tissues (Mocellin et al., 2001; Boni et al., 2010).

Metastatic renal cancer is another kind of tumor that is resistant to cytotoxic agents inherently and is responsive to cytokines like IFN-α and IL-2. Twenty five per cent response rate is observed in case of patients suffering from metastatic renal cancer when high dose of intravenous bolus of IL-2 is given and such response is durable (Fisher et al., 2000; Krieg et al., 2010). In case of advanced stages of renal cancer, IL-2 along with tyrosine kinase inhibitors is widely used for frontline therapeutic purpose (Lee and Margolin, 2011). IFN-β is produced by leukocytes and certain tumors and has got therapeutic potential in order to suppress autoimmune reactivity as well as for immunostimulating malignant tumor treatment in a number of preclinical studies. IFN-β is more effective than IFN-α in order to induce antiproliferative effects in cancer models (Johns et al., 1992; Chawla- Sarkar et al., 2001).

Combinations of cytokines help improve the cytokine-based cancer therapeutics. Combination therapy using IL-12 and granulocyte-macrophage colony stimulating factor (GM-CSF), administering two different plasmids that code for GM-CSF and IL-12, has been shown to induce strong anti-tumor effects, especially in hepato-cellular carcinomas (Wang et al., 2001; Stagg et al., 2004). Similarly, other workers have also described the combinations of GM-CSF/IL-2 and IL-12/IL-18, in promoting significant anti-neoplastic effects (Stagg et al., 2004). It has been suggested that the gene modification of mesenchymal stem cells (MSC) with therapeutic cytokines can augment the antitumor effects and this can help prolong the survival of tumor-bearing animals (Stagg et al., 2004; Hamadaet al., 2005). Further, role of cytokines in the upregulation of the cell signaling in neoplastic tissues has been a target in devising strategies to counter the tumor induction properties of certain cytokines, especially those involving the Ras-based pathways (Ancrile et al., 2008).

Molecular characterization of tumor antigens in melanoma provided the first support for the development of original clinical trials aimed to activate specific anti-tumor immune response. Mutated oncogenes, such as p53 represents an ideal tumor antigen since it is not present on normal cells. Successful immunization against this mutated protein was developed in a murine model only when IL-12 was used as adjuvant (Brunda et al., 1993). Similarly, fusion proteins which associate a tumor antigen and a cytokine could enhance the immunogenicity of antigen. For example, idiotypes of immunoglobulin's expressed in lymphoma when coupled with cytokines such as IL-2, IL-4, GM-CSF, and administered in mice were efficient to protect mice against the parental lymphoma (Chen et al., 1995). Therapeutic trials aimed to induce an immune response to the carcinoembryogenic antigen in colon cancer, the Muc1 antigen in breast cancer, and HPV encoded proteins in cervical cancer have been performed using vaccinia vector in which a cDNA encoding cytokine such as IL-2 or IL12 were also introduced (Peralta-Zaragoza et al., 2012).

Il-17/ IL-23 surrounding TH17 cells is an area of recent investigation as far as role of cytokines in autoimmune diseases and potentially in tumor immunity is concerned (Zou and Restifo, 2010). In human and mouse tumors, TH17 cells are assumed to play a role in microenvironment of the tumor (Zou and Restifo, 2010; Wilke et al., 2011) and in immuno-deficient mice IL-17 promote vascularization of tumors. Both pro-tumor as well as anti-tumor effects in different mouse models are shown by IL-23 and increase in the concentration of this particular cytokine at the site of tumor locally or even systemically leads to inhibition of tumor leading to inhibition of tumour and increase in

survivability (Langowski et al., 2006; Yuan et al., 2006; Kaiga et al., 2007).

As fusion proteins combining monoclonal antibodies with cytokines, immunocytokines were developed to improve upon the benefit: risk ratios of monoclonal antibodies and cytokines alone or as combination therapy (Cheng et al., 2000). Immunocytokines are hypothesized to confer two possible advantages over currently available immunotherapies. First, by providing both antibody effector activities and a cytokine co-signal for the generation of cytotoxic cellular immunity and thus may amplify antitumor immune responses relative to those obtained with current immunotherapies (Smyth et al., 2004). Second, by targeting delivery of cytokines to the tumor microenvironment, immunocytokines deliver biologically active concentrations of cytokines at lower and less toxic doses than are required by systemic cytokine therapy. Immunocytokines stimulate tumor-suppressing immune responses and their *in vivo* administration cause a greater antitumor effect than administration of a mixture of an equivalent dose of antibody and cytokine (Cheng et al., 2000; Mocellin et al., 2001; Cutler and Brombacher, 2005). The cytokine portion of the immunocytokine also provides a costimulatory signal for enhancing both NK-cell- and T-cell-mediated antitumor responses. Immunocytokines also appear to prolong cytokine biological activity relative to that of systemically administered cytokines. Several immunocytokines are being explored for potential use in human cancers, and the use of two (hu14.18-IL-2 and huKS-IL-2) gave promising results (Yang et al., 2012). Besides these, IL-2-based immunocytokines which have been thoroughly assessed in preclinical models and are undergoing evaluation in the clinic studies, IL-12 and granulocyte macrophage colony-stimulating factor-based (GM-CSF-based) immunocytokines are being assessed in preclinical tumor models (Varona and Villamayor, 2007).

Cytokines as vaccine adjuvants

Cytokines control the type and extent of an immune response following infection or vaccination and represent excellent naturally occurring therapeutics (Masihi, 2006; Varona and Villamayor, 2007). Cytokines initiate a cascade of events against infection and play important role leading to protective immune response. IL-1 induces receptor for IL-2 and CSFs, and cause leukocyte growth; differentiation, activation and synthesis of IL-2, 4, 6 and 8, activities which help in sustaining host immunity and inflammatory response (Tagliabue and Boraschi, 1993; Atkins, 2002; Kayamuro et al., 2010). The use of recombinant cytokines as vaccine adjuvants may offer myriad possibilities, whereby the magnitude and type of the immune response to vaccination can be willfully and specifically modified. The incorporation of cytokines as molecular adjuvants in vaccines has thus been tied to strengthen vaccine-induced immunity, and to modulate *in vivo* cytokine environment, by including T_h1 or T_h2 cytokines, so as to drive immune responses towards a desired type (Lofthouse et al., 1996; Chabalgoity et al., 2007; Varona and Villamayor, 2007). In mice, recombinant cytokines like IL-1, IL-2 and IFN-γ have been used primarily to enhance humoral responses. Cytokine adjuvant studies in ruminants revealed recombinant ovine and bovine IL-1 and IL-2 molecules to be beneficial. The effect of a cytokine adjuvant on bovine viral diarrhea virus (BVDV) DNA vaccine expressing the major glycoprotein-E2 has been attempted; IL-2 and GM-CSF showed potential ability to enhance the humoral and cellular immune responses involved in protection against BVD infection (Varona and Villamayor, 2007; Dhama et al., 2008c). The efficacy of DNA immunization can be improved by stimulating class-I and class-II MHC restricted T-lymphocytes, via the administration of cytokines (Dhama et al, 2008c). In poultry, the role of cytokines for improving DNA vaccine efficacy has been experimentally identified mainly for diseases like coccidiosis, infectious bronchitis, infectious bursal disease and avian influenza. Regarding helminth infections, cytokines such as IL-4 and IL-10 have been found to be more appropriate while being used along with DNA or subunit vaccines (Deng et al., 2001; Asif et al., 2004; Dhama et al., 2007, 2008c; Figueiredo et al., 2010).

Cytokines including type I IFNs and IFN-γ; IL-2 and 12 are responsible for the activities of various potent adjuvants *viz.,* incomplete Freund's adjuvant as well as CpG oligodeoxynucleotides and alum. IFN-α as well as IFN-γ; IL-2, 12, 15, 18 and 21; and GM-CSF and fetal liver tyrosine kinase (flt)-3 ligands potentially influence the immune response in a positive manner. One major problem however is the relatively short half life of the homologues of cytokines that are recombinant in nature which have been overcome by encapsulating with liposomes along with using expression vectors that are co-administered with DNA vaccines (Tovey and Lallemand, 2010). IL-1 in particular possesses adjuvant activity for a wide array of infectious as well as tumour antigens but the variety of side effects caused by the proinflammatory action of IL-1 represents a serious disadvantage as far as its use is concerned. IL-1β sequence has a non-peptide which is devoid of all proinflammatory activities but the immunostimulating activity of whole IL-1β is maintained and such specific sequence can be employed successfully in animals potentiating the immune response specifically against T-helper dependent cellular antigens (Tagliabue and Boraschi, 1993; Kayamuro et al., 2010).

As new adjuvants are required for the delivery of various vaccines, the role of cytokines has been evaluated as effective adjuvants (Thompson and Staats, 2011). Th1 cytokine: IL-12 induces natural killer (NK) cell activities along with T and B cell activities in order to produce IFN-γ and primarily engaged in differentiation of Th1 cells (Watford et al., 2003). As a nasal vaccine adjuvant IL-12 enhances serum antibody production (Bradney et al., 2002). Following co-delivery with two plasmids expressing HIV proteins plasmid-incorporating IL-2 when administered single time to mice causes significant decrease in serum anti-HIV immunoglobulin (Ig) G1 but increase in serum anti-HIV IgG2a (Xin et al., 1998, 1999). A different approach regarding the co-expression of IL-2 in *Lactococcus lactis* revealed that there is significant increase in serum IgG post primary immunization (Steidler et al., 1998). Study on delivery of IL-15 and DNA encoding herpesvirus glycoprotein B (gB) in mice model has proven the utility of IL-15 as vaccine adjuvant (Toka and Rouse, 2005). Type I IFN when co-delivered with influenza antigens provides nasal vaccine adjuvant activity (Proietti et al., 2002; Couch, 2004; Bracci et al., 2005; Longhi et al., 2009).

In murine as well as bovine, simian and human models the potential of GM-CSF as adjuvant has been evaluated extensively either for protection against viral (van Slooten et al., 2001) or bacterial challenges (Ding et al., 2004; Hovav et al., 2005). Plasmid expressing IL-4 when used as vaccine adjuvant has been found to be effective against herpes simplex virus (HSV) –1 induced ocular infection (Osorio and Ghiasi, 2003). Fusion protein between HIV glycoprotein (gp) 120 and IFN-γ plasmids was found to be effective for stimulating both antibody as well as T-cell responses (Nimal et al., 2005; Melchers et al., 2011).

Limitations of cytokine use

A number of factors have raised difficulties in adapting cytokines for safe and effective routine therapeutics for clinical practice. Need to uphold effective dose over a significant period of time is a major hurdle (Veltri and Smith, 1996). Achieving higher concentrations in pockets, when cytokines are administered systemically, is also another difficulty. In addition, cytokines have a very short half-life, so that continuous administration may be required; and they may also exert potent immune responses with unpredictable and undesirable side effects. Symptoms ranging from mild fever and chills to anemia, thrombocytopenia, respiratory distress, shock and coma, have been observed during administration of the cytokine IL-2. Despite these difficulties, the promise of cytokines for a practical implementation is great, and efforts to develop safe and effective cytokine-related therapeutic line of attack is going on, chiefly in treating infectious diseases and cancerous conditions, immunomodulation, and molecular adjuvants for vaccines.

To circumvent the major drawbacks of the practical applicability of cytokines as potent therapeutics, attempts have been made to increase half-life of cytokines by attaching the molecules to polyethylene glycol (PEG). Such PEG-ylated cytokines have shown an increase in their biological activity, which favors use of lower doses and dwindle down the risk of adverse reactions. Preparation of cytokine immunoassay references in basic as well as clinical research along with standards in order is the need of hour due to the increase in popularity of using cytokine immunoassays, but these immunoassay resulting from various sources must be compared with proper caution. In addition, understanding the biology of the systems under study is important with special reference to cross-reactivities with isoforms of cytokines as well as soluble receptors' inferences and matrix effect on samples of interest. Proper precautionary measures if unavailable become a serious issue for immunoassay developers while selecting unbiased antibodies against recombinant forms of cytokines (Tsang and Weatherbee, 1996; Deb et al., 2013). Problem of the relatively short half life of the recombinant cytokine homologues is being overpowered by liposome encapsulation along with using expression vectors co-administered with DNA vaccines (Dhama et al., 2008c; Tovey and Lallemand, 2010).

Systemic cytokine therapy has limitations viz., rapid cytokine degradation and elimination, attaining optimal concentrations in the tumor microenvironment is difficult, dose dependent toxicity putting life-threatening side effects such as vascular leak syndrome and orthostatic hypotension. Targeted delivery of immunocytokines to the tumor microenvironment could deliver biologically active cytokine concentrations at lower and less toxic doses compared to systemic delivery. Cytokine gene therapy, in which a cytokine gene (such as that for IL-2) is introduced into tumor cells, is therefore being explored to overcome some of the limitations of systemic cytokine administration (Mocellin et al., 2001; Nixon et al., 2007). However, cytokine gene therapy is technologically challenging and resource-intensive.

Conclusion and future perspectives

Cytokine therapy is a natural alternative for disease control. Cytokines play an essential role in inter-cellular communication, and are considered vital to the mediation and regulation of inflammatory or immune responses, and are thought of as highly suitable candidates for various therapeutic options in human as well as animal health care. Developing the recombinant cytokines and exploiting them for modulating immune

responses is a staggering task, as their functions are complex and the effects are dependent on length of exposure, target cells and the presence of other cytokines in the same environment. A major limitation in the use of cytokine-based immunotherapies is their extraordinarily short half-life *in vivo*, and side effects. A novel approach for increasing half-life of cytokines is covalent attachment of the cytokine to polyethylene glycol which can extend their biological activity, thus reducing the required doses and as well as limiting the concerned risks of adverse affects/reactions. For the wide use and multiple applications of these highly pleiotropic beneficial molecules, in depth understanding of cytokine homeostasis is required. The incorporation of cytokines as molecular adjuvants in vaccines or as effector molecules for prevention or treatment of human neoplastic conditions or infectious diseases is a hot topic amongst researchers and clinicians and need to be suitably investigated and revised in relation to the potent applications in animal and veterinary sciences. Immunocytokines have good potentials for improving outcomes for patients with cancer. As administration of immunocytokines poses no apparent risk of cross-resistance with conventional therapeutic modalities including chemotherapeutics, radiotherapy, and surgery, combination therapy appears to be feasible and should be explored. Hence, combination therapy with cytokines in particular, may be a fruitful approach demonstrating synergy of immunocytokines and standard chemotherapies along with immuno-modulatory approaches such as gene therapy which may help fighting the deadly malady like cancers. The use of cytokines for the purpose of intra-nasal vaccination is a matter of concern and ongoing research reflect their future uses to provide with effective adjuvant activities. The utilization of cytokines is becoming more feasible with the recent cloning of a number of cytokine genes and the establishment of commercially feasible methods of delivery. The rapid research developments in the field of cytokine therapeutics and its allied applications reveal beyond doubt that their use in veterinary medicine won't be a distant dream for better prospects in the field of animal disease prevention and therapeutics, in the years to come.

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