Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry, 2008, *7*, 122-129

Feasibility and Prospects for Anti-Inflammatory Antibodies in the Treatment and Disease Management of Influenza

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INTRODUCTION

Influenza, commonly known as flu, is an infectious disease of birds and mammals caused by an RNA virus of the family Orthomyxoviridae (influenza viruses). Influenza viruses are classified as type A, B, and C based upon their protein composition [1]. The influenza A virus is the most frightening pathogen among the three and is a major public threat [2]. It was responsible for the global outbreaks of 1918, 1957 and 1968 [3-5]. The influenza virus attacks the upper respiratory tracts of humans in all age groups and might cause acute lung inflammation, which is an important cause of morbidity and mortality, particularly in children, elderly people and those with chronic diseases [1, 6]. Influenza results in over 100,000 people being hospitalized annually and around 36,000 deaths each year in the USA. There is tremendous concern that highly virulent variants of the influenza virus may emerge to which humans have little immunity, resulting in a devastating pandemic [7-10].

Studies on the pathogenesis of influenza have shown that the lethal effect of the influenza virus results from lung inflammation of the host rather than from the direct cytopathy of viral replication [11-13]. The pathological events in the virulent influenza are illustrated in Fig. **1**. When the influenza virus attacks the respiratory tract, it infects not only the epithelial cells of the tract, but also macrophages and other leukocytes [14]. Virus replication in these cells leads to the destruction of the cells [15, 16] and induces an antiviral response. This induces production and secretion of interferon (IFN)-α/β, which has direct antiviral effects, and a variety of proinflammatory cytokines [interleukin (IL)-1α/β, IL-6, IL-8, IL-18, IFN-γ, tumor necrosis factor (TNF)-α] and chemokines [regulated on activation, normal T cell expressed and secreted (RANTES), monocyte chemo-

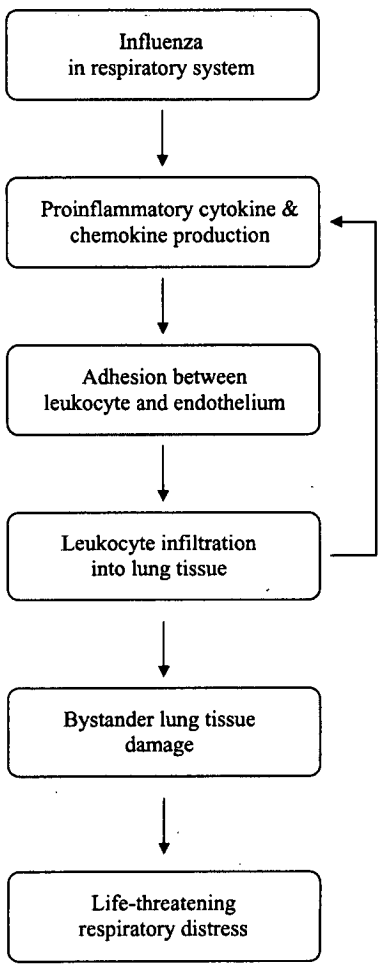

Fig. (1). Sequence of pathological events in virulent influenza

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attractant protein (MCP-1), MCP-3, IFN-γ-inducible protein-10 (IP-10), macrophage inflammatory protein (MIP)-1α/β] [17-28]. In an effort to contain viral infection, these proinflammatory cytokines and chemokines trigger a rapid inflammation in the lung, including swelling of localized blood capillaries, the movement of fluid and leukocytes into lung tissue and activation of infiltrated leukocytes. However, certain virulent strains, such as avian influenza virus type A, H5N1 strain are resistant to the antiviral effects of these proinflammatory cytokines and chemokines [29]. The more virulent influenza virus strains are, the more likely they are to trigger the production of proinflammatory cytokines and chemokines and recruitment of leukocytes into the lung. Consequently, inflammation can be overexuberant in the lung [18, 19]. The overexuberant inflammation results in bystander lung tissue damage and lung filling with fluid and debris, which makes it increasingly hard to breathe. This might indeed contribute to the unusual severity of the disease caused by H5N1 strain in humans, which can occlude the airway so as to escalate into life-threatening pneumonia and acute respiratory distress.

Inflammation is a defensive response that begins after infections to eliminate infectious agents. The body definitely needs a certain amount of inflammation to protect itself against diseases. However, what the body wants with any infection is a proper amount of inflammation to recruit inflammatory cells to fight it off, but not so much that it starts being harmful to local normal organs or even to the whole body by so-called "cytokine storm"[30]. The role of inflammation in the influenza virus-induced lung damage is supported by studies in which anti-inflammatory treatment deceased lung damage and mortality in influenza-infected mice [31]. This has led to the concept that attenuation of inflammation early in the disease process might improve the outcome.

Antibodies (Abs) with high specificity and affinity can function in many ways by neutralizing/blocking target molecules or by marking cells for destruction by effector cells. Therefore, Abs can be developed to bind to specific proinflammatory cytokines, chemokines, leukocyte adhesion molecules or their receptors to inhibit the effects of these proinflammatory cytokines, chemokines and adhesion molecules so as to attenuate the host overexuberant inflammation in the lung during virulent influenza. However, application of these Abs to attenuate lung inflammation in virulent influenza has been rarely reported, although quite a few studies have been reported about anti-inflammatory Abs for treatment of inflammatory diseases such as Crohn's disease (CD), ulcerative colitis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis and psoriasis [32-35]. This review focuses on anti-inflammatory Abs and discusses the feasibility and prospects for using them to attenuate the host inflammatory responses in the lung for the treatment and disease management of virulent influenza.

FORMATS OF ANTI-INFLAMMATORY ABS

Chimeric, Humanized, and Fully Human Abs

Murine monoclonal Abs (mAbs) have serious disadvantages as therapeutic agents in humans [36,37]. They induce human anti-mouse Ab (HAMA) responses [38]. Repeat administration of murine mAbs may result in rapid clearance of the murine mAbs and anaphylaxis, which can sometimes be fatal [39, 40]. Using hybridoma methods to immortalize human B-cells would avoid this issue, but is problematic owing to the absence of a suitable fusion partner for human B cells and other technical issues [41]. To overcome this hurdle, chimeric or "hybrid Abs" can be constructed by linking human Ab heavy and light chain constant regions (CH, CL) with murine corresponding variable regions (VH, VL) using a DNA engineering approach [42-44] (Fig. 2). Although chimeric Abs are less foreign and therefore less immunogenic than mouse mAbs, human anti-chimeric Ab (HACA) responses have still been observed [45, 46]. To further minimize the mouse components of Abs, humanization of murine mAbs should be implemented, in which only the complementarity-determining region (CDR) loops that are responsible for antigen binding are grafted onto the human variable region frameworks with human CH and CL [47-49] (Fig. 2). These humanized Abs displayed better performance in clinical trials [50, 51]. However, human anti-humanized Ab (HAHA) responses still exist, although with a reduced incidence compared with HAMA and HACA. Modern alternative strategies now allow development of fully human Abs directly from phage-display libraries of human Ab fragments [52]. Another approach is to use mice that are transgenic for the human Ig locus [53]. Immunization of such a transgenic mouse leads to the development of human Abs, from which hybridomas that produce human Abs can be generated.

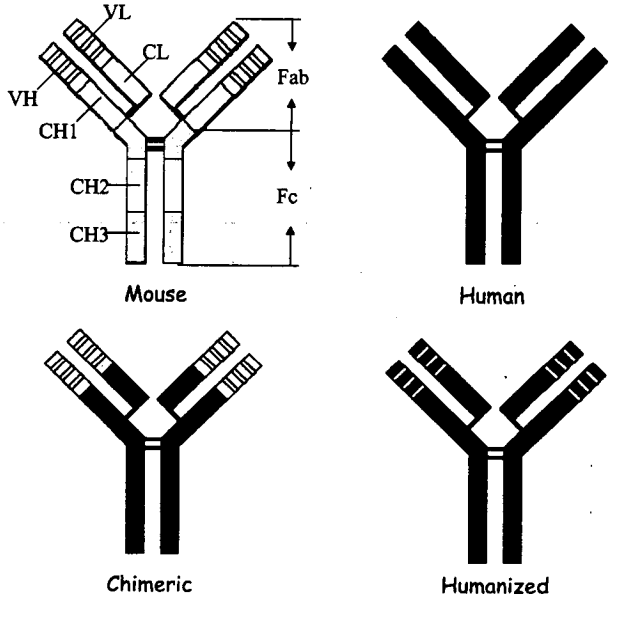

Fig. (2). Antibody structure and formats

Antigen-Binding Fragment (Fab)

Abs consist of two Fabs and one fragment crystallizable region (Fc) (Fig. 2). Fabs bind to antigens and Fc binds to various effector molecules of the immune system. Therapeutic Abs do not always need to recruit effector cells or activate complement for efficacy. Fabs can do part of the job of intact Abs, such as blocking the interaction between a cytokine and its receptor. As an anti-inflammatory Ab, in fact, Ab effector

functions such as activation of complement and resultant release of inflammatory mediators are not only unnecessary, but also harmful. Therefore, Fabs are a good format to be therapeutic agents for inflammatory diseases.

Long Half-Life Abs

Although Fabs are better than whole Abs in some ways for treatment of inflammatory diseases, Fabs show fast off-rates and poor retention time in the circulation, and as a result the fraction of the administration dose that reaches its target is too low for a therapeutic benefit. Therefore, the manipulation of Fabs to increase their half-lives will be crucial for the success in the treatment of inflammatory diseases. Complete human Abs have long serum half-lives owing to their ability to bind to the so-called neonatal Fc receptor. Although the sequences of Fc of the four IgG subtypes are very similar, they differ markedly in their Abs to recruit effector functions. IgG1 and IgG3 are very effective in recruiting effector functions, whereas IgG2 is less effective and IgG4 is nearly ineffective. Therefore, Fabs can be grafted on the respective Fc of a human IgG4 in order to decrease the risk for recruitment of effector functions and to increase the half-life of the Ab. CDP571 is a humanized anti-TNF-α IgG4 Ab, which showed a improved half-life in humans. The short half-life of Fabs can also be extended by "pegylation", that is, a fusion to polyethylene glycol (PEG) [54]. The effect is achieved by chemical coupling of PEG to amino groups in the protein structure to increase the size of the molecule above the glomerular-filtration limit. Anti-TNF-α human Fab (CD870; Celltech) had its circulating half-life prolonged to two weeks by site-specific pegylation in the hinge region [55].

ABS AS LEUKOCYTE AND EPITHELIUM ADHESION BLOCKERS

The leukocyte infiltration into lung tissue is a key event in lung inflammation during influenza. Many adhesion molecules play an important role in trafficking leukocytes into the inflamed lung tissues and they are up-regulated in the lung during influenza. The process of leukocyte infiltration in lung inflammation involves in a series of steps (Fig. 3): proinflammatory cytokines upregulate or induce the adhesion molecule expression on the vascular endothelium and leukocytes so as to cause leukocyte-endothelial cell adhesion (rolling, and sticking); chemokines bind to their specific cell surface receptors in leukocytes to cause a rapid change in leukocyte shape and behavior enabling the cells to transmigrate and migrate beyond the vascular barrier along a leukocyte-specific chemotactic gradient formed by chemokines [56]. Some important adhesion molecules have been found, such as selectins, integrins, intercellular adhesion molecule-1(ICAM-1), and vascular adhesion protein-1 (VAP-1) in leukocyte filtration process. They mediate the receptor-dependent interaction of leukocytes along the endothelium. Blocking these adhesion molecules might inhibit leukocyte interactions with the vascular endothelium, subsequently preventing downstream events including extravasation of leukocytes into tissues. This, in turn, reduces the bystander tissue damage and airway obstruction from recruited leukocytes during inflammatory processes in the lung.

Selectins are a family of cell-surface adhesion molecules of leukocytes (L-selectin), endothelial cells (E-selectin), and platelet (P-selectin) [57,58]. Selectins are involved in the first step of leukocyte transmigration during inflammation, that is, leukocyte rolling (Fig. 3) [59]. Each of the three selectins can mediate leukocyte rolling in the appropriate conditions. LigoCyte, a pharmaceutical company (www. ligocyte. com), found that the inhibition of both E-selectin and L-selectin by a mAb, known as EL-246, in large animal models (sheep, pig, or primate) of pulmonary infection with pseudomonas resulted in a reduction of neutrophil and macrophage recruitment to the lung and administration of the mAb did not increase the opportunity for infections. Rather, the infections appeared to clear more rapidly with an attenuated inflammatory response.

Integrins contain large (α) and small (β) subunits. Mammalian integrins form several subfamilies sharing common β subunit that associates with different α subunit. Integrins on the surface of leukocytes are involved in leukocyte sticking to the endothelial cells after leukocyte rolling (Fig. 3). Tysabri (http://www.tysabri.com), a humanized mAb, bound to α4 subunit of α4β1 and α4β7 integrins expressed on the surface of all leukocytes except neutrophils, and inhibited the α4 integrins-mediated adhesion of leukocytes to endothelial cells. Another integrin, αDβ2 is also expressed on leukocytes, such as monocytes/macrophages and neutrophils. Administration of an anti-αD subunit (CD11d) mAb would effectively reduce neutrophil and macrophage infiltration into lesions by 70% and 36% respectively so as to attenuate the inflammatory response after a spinal cord transaction injury in rats [60, 61].

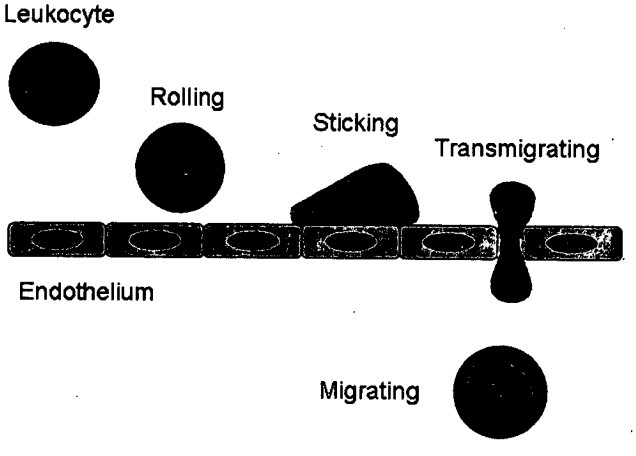

Fig. (3). The process of leukocyte infiltration in lung inflammation during influenza

ICAM-1 belongs to immunoglobulin superfamily cellular adhesion molecules (CAM) [62,63]. ICAM-1 on the surface of endothelial cells is the main ligand responsible for leukocyte adhesion mediated by β2 integrins. An anti-ICAM-1 Ab was demonstrated to reduce virus-induced increases of neutrophils and lymphocytes in bronchial alveolar liquid (BAL) significantly by 70% ($P<0.001$) [64]. Another anti-ICAM-1 Ab was reported to significantly inhibit pulmonary inflammation to reduce idiopathic pneumonia syndrome, which is a

frequently fatal complication of allogeneic bone marrow transplantation in a murine model [60].

VAP-1 is on the endothelial cell membrane, which is integrin receptors and mediates specific binding of CD8 T cells, as well as natural killer (NK) cells to endothelial cells in lymph nodes and at sites of inflammation [65]. The blockage of VAP-1 by a mouse-human chimeric anti-VAP-1 Ab remarkably reduced leukocyte adhesion and transmigration into tissues *in vivo* and *in vitro* models [66], suggesting that VAP-1 is a potential target for anti-inflammatory therapy and anti-VAP-1 Ab has the potential to be used as a new anti-inflammatory therapy agent.

ABS AS PROINFLAMMATORY CYTOKINE OR CHEMOKINE INHIBITORS

Proinflammatory cytokines and chemokines play a key role in pathophysiology of lung inflammation during influenza [23,28]. A wide variety of proinflammatory cytokines and chemokines orchestrate the inflammatory response [28]. Each of them has specific, but often overlapping functions. They exert their effects through G-protein coupling receptors expressed on inflammatory cells. Therefore, inhibition of these proinflammatory cytokines and chemokines through blocking the binding of proinflammatory cytokines and chemokines to their receptors by anti-proinflammatory cytokine and anti-chemokine Abs may have therapeutic potential.

1. Anti-TNF-α Abs

TNF-α is a major proinflammatory cytokine, which is abundantly expressed not only in influenza, but also in other inflammatory diseases [28, 67,68]. In an animal model of influenza A virus A/PR/8/34 (H1N1) infection, anti-TNF-α Abs have been demonstrated to be effective in the suppression of inflammation and improve survival rate [69]. TNF-α has also been validated as a good target for treatment of some other inflammatory diseases including CD, ulcerative colitis and rheumatoid arthritis. Several anti-TNF-α Abs have thus been developed. These Abs include infliximab [33, 70], adalimumab [52], CDP571, and CDP870 [55, 71].

Infliximab is the most studied and the first anti-TNF-α Ab licensed by the Food and Drug Administration (FDA) for clinical use. The Ab neutralizes the biological activity of TNF-α by binding with high affinity to the soluble and transmembrane forms of TNF-α and inhibits or prevents the effective binding of TNF-α with its receptors. It has been demonstrated to be effective in both induction and maintenance therapy for inflammatory diseases [33, 70] However, infliximab is a mouse-human chimeric anti-TNF-α Ab. Because of the potential for an immune reaction to the mouse protein components of a chimeric Ab, an alternate strategy has been used to develop humanized or fully human anti-TNF-α Abs.

Adalimumab is a fully humanized anti-TNF-α Ab and has received FDA approval for treatment of some inflammatory diseases [52]. It is as efficacious as infliximab for treatment of inflammatory diseases. As adalimumab does not contain a mouse peptide sequence, it is expected to be less immunogenic and more tolerable than infliximab.

CDP571 and CDP870 are other two kind of humanized anti-TNF-α Abs [55, 71]. The former is a humanized IgG4 Ab, made by genetic engineering to replace the murine frameworks with counterparts of a human IgG4. The latter is a pegylated (linked to be polyethylene glycol) Fab of humanized Ab. Both of them were designed to have less Ab effector function and a prolonged plasma half-life. Both anti-TNF-α Abs have been proven to be effective in treatment of some inflammatory diseases [55, 71] .

2. Anti-IFN-γ Ab

One of the hallmarks of influenza is the induction of IFN-γ by a wide range of cell types [72]. IFN-γ has been identified as an important inflammatory mediator [73]. *In vivo* neutralization of IFN-γ led to a significant reduction in the magnitude of the cellular infiltration in the lung tissue after influenza [74]. Fontolizumab is a humanized anti-human IFN-γ Ab [75]. It has shown excellent safety and tolerability in a phase I/II, double blind, placebo controlled, single and multiple dose, dose escalation study in patients with moderate to severe inflammation of CD [76].

3. Anti-IL-8 Ab

IL-8, a CXC chemokine, is a powerful chemotactic factor and activator for neutrophils. Neutrophilic inflammation is a major feature of chronic obstructive pulmonary disease (COPD). Pretreatment of neutrophils with an anti-IL-8 Ab led to a concentration-dependent inhibition of sputum-induced neutrophil chemotaxis [77].

4. Anti-MCP-1 Abs

MCP-1, a chemokine, can recruit leukocytes to sites of infection and inflammation by activation of chemokine CC receptor 2 (CCR2) on monocytes and T lymphocytes. In addition, this chemokine is also a potent histamine-releasing agent for basophils and mast cells [78-81]. In a mouse model of asthma with blocking MCP-1 by an anti-MCP-1 Ab, there was a ~50 % reduction in the number of lung monocytes/macrophages, but interestingly, there was an almost complete reduction in lymphocyte and eosinophil infiltration of the lung. Consequently, bronchial hyperresponsiveness and lung inflammation were diminished drastically [82]. Furthermore, the neutralization of MCP-1 by an anti-MCP-1 polyclonal Ab inhibited changes in airway resistance and attenuated histamine release into the BAL in a murine model of cockroach antigen-induced allergic airway inflammation [83].

5. Anti-RANTES Abs

RANTES is chemotactic for T cells, eosinophils and basophils and plays an active role in recruiting leukocytes into inflammatory sites. In a murine model of respiratory syncytial virus infection, treatment with an anti-RANTES Ab demonstrated significant decrease in airway hyperreactivity (AHR) [84]. Another study demonstrated neutralization of RANTES with an anti-RANTES Ab remarkably attenuated mononuclear cell recruitment during episodes of AHR in a rat model of lung allograft rejection [85].

ABS AS INFLAMMATORY CYTOKINE OR CHEMOKINE RECEPTOR ANTAGONISTS

Proinflammatory cytokines or chemokines bind their cell-surface receptors to exert their effects by subsequent cascades of intracellular signaling and then alter cell functions. Therefore, cytokine and chemokine receptor antagonists can inhibit cytokine or chemokine biological functions by competitive binding to their receptors.

1. Anti-IL-1 Receptor Ab

IL-1, like TNF-α and IL-6 is an endogenous pyrogen. It contributes to leukocytosis by release of neutrophils from the bone marrow and induces the production of other proinflammatory cytokines and chemokines including IL-6, IL-8, RANTES, GM-CSF, and TNF-α from a variety of cells. IL-1 can also induce the expression of the adhesion molecules on endothelial cells, which lead to the increased edhesion of neutrophils and eosinophils to the vascular endothelium and respiratory epithelium [86]. An anti-IL-1 receptor mAb 35F5 has been shown to inhibit the ability of IL-1 to bind to IL-1 receptor. Additionally, the mAb 35F5 inhibited a variety of inflammatory responses *in vitro* and *in vivo*. Specific *in vivo* blockade of IL-1's action with this mAb attenuated the host inflammatory response [86].

2. Anti-IL-6 Receptor Ab

IL-6, proinflammatory cytokine can transduce signals into cells without IL-6 receptor expressed on the cell membrane when IL-6 binds to soluble IL-6 receptor. A humanized anti-human IL-6 receptor Ab, called MRA, Actemra, or Tocilizumab, was developed for treatment of systemic inflammatory disease, such as rheumatoid arthritis and systemic-onset juvenile idiopathic arthritis. In Phase III studies, the Ab has shown efficacy as a monotherapy in inhibiting the progression of joint destruction in systemic inflammatory diseases [87].

3. Anti-CCR2 Ab

Recruitment and activation of leukocytes by chemokines depends on the expression of specific cell surface receptors. There are approximately two dozen chemokine receptors [88]. Most of these receptors belong to one of two major subfamilies based on the arrangement of two of the first four cysteine residues in the protein's molecule—the CXC chemokines and the CC chemokines. Some chemokine receptors appear to be selective for single chemokines, whereas others are promiscuous and mediate the effects of several related chemokines. Because of this, these receptors seem like ideal targets for the challenging field of anti-inflammatory agent development.

The CCR2 of which primary ligand is MCP-1, is the most popular chemokine receptor under study. MLN1202, a humanized Ab developed by Millennium Pharmaceuticals (http://www.mlnm.com), targets the CCR2 and is a CCR2 antagonist in Phase II trials for rheumatoid arthritis, multiple sclerosis and atherosclerosis.

ABS AS PROINFLAMMATORY CYTOKINE AND CHEMOKINE PRODUCTION INHIBITORS (TOLL-LIKE RECEPTOR ANTAGONISTS)

Although there are several attempts to block some proinflammatory cytokines and chemokines or their receptors as discussed above, this may not be enough to control lung inflammation in virulent influenza, since so many proinflammatory cytokines and chemokines are involved and this is considerable redundancy in their effects [28, 31]. This suggests that development of some Abs that have a more general effect on inhibition of proinflammatory cytokine and chemokine synthesis may be more promising.

Proinflammatory cytokines or chemokines are produced by activation of toll-like receptors (TLR) [89, 90]. TLR have recently emerged as key receptors in almost all cells of the body. They are responsible for recognizing specific conserved components of microbes to provoke innate immunity and to establish adaptive immunity. To date, 10 TLRs (TLR 1 to TLR 10) have been reported in human [91]. However, each TLR has common effects, such as eliciting proinflammatory cytokine and chemokine production and upregulating costimulatory molecule expression via activation of the NF-κB pathway. These immunoadjuvant effects are not only critical in antimicrobial immunity but are also involved in manifestations of overexuberant inflammation and autoimmunity. Different TLRs recognize different microbes (Table 1). Most of TLRs are expressed on the cell surface; however, a subset of TLR3, TLR7, TLR8 and TLR9 are retained in intracellular compartments, endosome. They appear to play important roles in responses to viruses [92]. To date, TLRs 3, and 7 have been found to mediate responses to influenza viral RNA [93, 94]. TLR-3 was found to play a key role in lung inflammation and lethality in a mouse model of influenza, indicating TLR-3 antagonists might down-regulate overexuberant inflammation during influenza [95]. TLR 2 and 4, the principal receptors involved in recognition of various bacterial cell wall components, have been most intensively studied for inhibition of cytokine production using anti-TLR 2 and 4 Abs [96-99]. Although there are some antiviral TLR Abs, there have not been any research reports which showed that these kind of Abs could inhibit proinflammatory cytokines and chemokine production induced by viruses. This might be partly due to the fact that all virus-related TLRs are located on the membrane of endosome in the cytoplasma, not on the cell surface. Therefore, study on inhibition of virus-driven proinflammatory cytokine and chemokine production by anti-viral TLR Abs is more complicated. Successful delivery of anti-viral TLR Abs into cytoplasm is prerequisite to study the inhibition of virus-driven proinflammatory cytokine and chemokine production by anti-viral TLR Abs. To date, there are a couple of delivery systems available to transport functional proteins into living cells. The most actively studied approach is "protein transduction domains" [100] or membrane transport signals" [101]. They are a class of peptides comprising 10-35 positively changed amino acids, which are potentially important for contact with cell. A cationic lipid formulation (TFA-DODAPL:DOPE) has also been showed to delivery a number of different macromolecules including Abs into the cyto-

plasm of numerous cell types. Nevertheless, viral TLR blockage for inhibition of virus-driven proinflammatory cytokine and chemokine production by antiviral TLR Abs might be worth studying.

Table 1. Toll-Like Receptors and Their Known Microbial Ligands [89, 91, 92]

TLR Family	Microbial Ligands
Cell Surface TLRs	
TLR1/TLR2 pair	tri-acyl lipopeptides (bacteria, mycobacteria), lipoarabinomannan (myobacteria)
TLR2/TLR6 pair	lipoteichoic acid from gram-positive bacteria cell walls
TLR4	liposacchrides from gram-negative bacteria cell walls
TLR5	flagellin (bacteria)
TLR10	unknown
Endosome TLRs	
TLR3	dsRNA (viruses)
TLR7	ssRNA (viruses)
TLR8	ssRNA (viruses)
TLR9	unmethylated CpG (bacteria or viruses)

CONCLUSIONS

Reduction in the mortality rate of virulent influenza, not only depends on the improved antiviral agents, but also requires the development of some agents to attenuate overexuberant lung inflammation. Although it has rarely been reported that Abs were used as anti-inflammatory agents to attenuate overexuberant lung inflammation during influenza, there are encouraging clinic data to show that Abs are good agents with blocking activity to inhibit adhesion molecules, proinflammatory cytokines, and chemokines to change vascular permeability, leukocyte chemotaxis, leukocyte activation and hyperthermia in treatment of inflammatory diseases. Advances in modern molecular and biotechnological approaches have facilitated abundant production of high-quality therapeutic anti-inflammatory Abs for attenuating inflammation. Therefore, co-administration of effective antiviral agents with these Abs as inflammation attenuators, by which a reduction, but not an elimination of inflammation could improve the lung function without compromising virus clearance, and might result in a better treatment outcome of virulent influenza.

Abbreviations:

AHR = Airway hyperreactivity

Abs = Antibodies

Fab = Antigen-binding fragment

BAL = Bronchial alveolar liquid

CCR2 = CC receptor 2

CAM = Cellular adhesion molecules

COPD = Chronic obstructive pulmonary disease

CDR = Complementarity-determining region

CD = Crohn's disease

FDA = Food and Drug Administration

Fc = Fragment crystallizable region

CH = Heavy chain constant regions

VH = Heavy chain variable regions

HACA = Human anti-chimeric Ab

HAHA = Human anti-humanized Ab

HAMA = Human anti-mouse Ab

IP-10 = IFN-γ-inducible protein-10

ICAM-1 = Intercellular adhesion molecule-1

IFN = Interferon

IL = Interleukin

CL = Light chain constant regions

VL) = Light chain variable regions

MIP) = Macrophage inflammatory protein

mAbs = Monoclonal antibodies

MCP-1 = Monocyte chemoattractant protein

NK = Natural killer

PEG = Polyethylene glycol

RANTES = Regulated on activation, normal T cell expressed and secreted

TLR = Toll-like receptors

TNF = Tumor necrosis factor

VAP-1 = Vascular adhesion protein-1

REFERENCES

[1] BR Murphy, W. R. Orthomyxoviruses, Raven press, NewYork **1990**.

[2] Niederman, M. S.; Mandell, L. A.; Anzueto, A.; Bass, J. B.; Broughton, W. A.; Campbell, G. D.; Dean, N.; File, T.; Fine, M. J.; Gross, P. A.; Martinez, F.; Marrie, T. J.; Plouffe, J. F.; Ramirez, J.; Sarosi, G. A.; Torres, A.; Wilson, R.; Yu, V. L. *Am. J. Respir. Crit. Care Med.*, **2001**, *163*, 1730-54.

[3] Hsieh, Y. C.; Wu, T. Z.; Liu, D. P.; Shao, P. L.; Chang, L. Y.; Lu, C. Y.; Lee, C. Y.; Huang, F. Y.; Huang, L. M. *J. Formos Med. Assoc.*, **2006**, *105*, 1-6.

[4] Linder F.E.; Grove, R. D. (1943) Washington, DC: Government Printing Office.

[5] Simonsen, L.; Clarke, M. J.; Schonberger, L. B.; Arden, N. H.; Cox, N. J.; Fukuda, K. *J. Infect. Dis.*, **1998**, *178*, 53-60.

[6] Barker, W. H.; Mullooly, J. P. *Arch. Intern. Med.*, **1982**, *142*, 85-9.

[7] Nguyen-Van-Tam, J. S.; Hampson, A. W. *Vaccine*, **2003**, *21*, 1762-8.

[8] Horimoto, T.; Kawaoka, Y. *Clin. Microbiol. Rev.*, **2001**, *14*, 129-49.

[9] Simonsen, L.; Fukuda, K.; Schonberger, L. B.; Cox, N. J. *J. Infect. Dis.*, **2000**, *181*, 831-7.

[10] Thompson, W. W.; Shay, D. K.; Weintraub, E.; Brammer, L.; Cox, N.; Anderson, L. J.; Fukuda, K. *JAMA*, **2003**, *289*, 179-86.

[11] Hurd, J.Heath, R. B. *Infect. Immun.*, **1975**, *11*, 886-9.

[12] Sullivan, J. L. Mayner, R. E. Barry, D. W.Ennis, F. A. *J. Infect. Dis.*, **1976**, *133*, 91-4.

[13] Shimomura, E.; Suzuki, F.; Ishida, N. *Microbiol. Immunol.*, **1982**, *26*, 129-38.

[14] Ronni, T.; Matikainen, S.; Sareneva, T.; Melen, K.; Pirhonen, J.; Keskinen, P.; Julkunen, I. *J. Immunol.*, **1997**, *158*, 2363-74.

[15] Lamb R, K. R. (1996) Orthomyxoviridae: the viruses and their replication., Lippincott-Raven Publishers, Philadelphia.

[16] Fesq, H.; Bacher, M.; Nain, M. Gemsa, D. *Immunobiology*, **1994**, *190*, 175-82.

[17] Adachi, M.; Matsukura, S.; Tokunaga, H. Kokubu, F. *Int. Arch. Allergy Immunol.*, **1997**, *113*, 307-11.

[18] Chan, M. C.; Cheung, C. Y.; Chui, W. H.; Tsao, S. W.; Nicholls, J. M.; Chan, Y. O.; Chan, R. W.; Long, H. T.; Poon, L. L.; Guan, Y.; Peiris, J. S. *Respir. Res.*, **2005**, *6*, 135.

[19] Cheung, C. Y.; Poon, L. L.; Lau, A. S.; Luk, W.; Lau, Y. L.; Shortridge, K. F.; Gordon, S.; Guan, Y. Peiris, J. S. *Lancet*, **2002**, *360*, 1831-7.

[20] Cook, D. N.; Beck, M. A.; Coffman, T. M.; Kirby, S. L.; Sheridan, J. F.; Pragnell, I. B.; Smithies, O. *Science*, **1995**, *269*, 1583-5.

[21] Hofmann, P.; Sprenger, H.; Kaufmann, A.; Bender, A.; Hasse, C.; Nain, M. Gemsa, D. *J. Leukoc. Biol.*, **1997**, *61*, 408-14.

[22] Julkunen, I.; Melen, K.; Nyqvist, M.; Pirhonen, J.; Sareneva, T. Matikainen, S. *Vaccine*, **2000**, *19* Suppl 1, S32-7.

[23] Lipatov, A. S.; Andreansky, S.; Webby, R. J.; Hulse, D. J.; Rehg, J. E.; Krauss, S.; Perez, D. R.; Doherty, P. C.; Webster, R. G. Sangster, M. Y. *J. Gen. Virol.*, **2005**, *86*, 1121-30.

[24] Matikainen, S.; Pirhonen, J.; Miettinen, M.; Lehtonen, A.; Govenius-Vintola, C.; Sareneva, T. Julkunen, I. *Virology*, **2000**, *276*, 138-47.

[25] Matsukura, S.; Kokubu, F.; Noda, H.; Tokunaga, H. Adachi, M. *J. Allergy Clin. Immunol.*, **1996**, *98*, 1080-7.

[26] Peschke, T.; Bender, A.; Nain, M. Gemsa, D. *Immunobiology*, **1993**, *189*, 340-55.

[27] Pirhonen, J.; Sareneva, T.; Kurimoto, M.; Julkunen, I. Matikainen, S. *J. Immunol.*, **1999**, *162*, 7322-9.

[28] Julkunen, I.; Sareneva, T.; Pirhonen, J.; Ronni, T.; Melen, K. Matikainen, S. *Cytokine Growth Factor Rev.*, **2001**, *12*, 171-80.

[29] Seo, S. H.; Hoffmann, E.; Webster, R. G. *Nat. Med.*, **2002**, *8*, 950-4.

[30] Yuen, K. (2005) *in* "Behind the Medical Headlines", Vol. 2005, the Royal College of Physicians of Edinburgh, Royal College of Surgeons of Edinburgh and Royal College of Physicians and Surgeons of Glasgow.

[31] Van Reeth, K. *Vet. Microbiol.*, **2000**, *74*, 109-16.

[32] Chikanza, I. C. *Paediatr. Drugs*, **2002**, *4*, 335-48.

[33] Breedveld, F. C. *Rheumatology (Oxford)*, **1999**, *38* Suppl 2, 11-3.

[34] Sandborn, W. J.; Hanauer, S. B. *Inflamm. Bowel Dis.*, **1999**, *5*, 119-33.

[35] Papoutsaki, M.; Chimenti, M. S.; Costanzo, A.; Talamonti, M.; Zangrilli, A.; Giunta, A.; Bianchi, L.; Chimenti, S. *J. Am. Acad. Dermatol.*, **2007**, *57*, 269-75.

[36] Breedveld, F. C. *Lancet*, **2000**, *355*, 735-40.

[37] Schroff, R. W.; Foon, K. A.; Beatty, S. M.; Oldham, R. K.; Morgan, A. C.; Jr. *Cancer Res.*, **1985**, *45*, 879-85.

[38] Khazaeli, M. B.; Conry, R. M.; LoBuglio, A. F. *J. Immunother.*, **1994**, *15*, 42-52.

[39] Hasholzner, U.; Stieber, P.; Meier, W.; Lamerz, R. *Anticancer Res.*, **1997**, *17*, 3055-8.

[40] Kuus-Reichel, K.; Grauer, L. S.; Karavodin, L. M.; Knott, C.; Krusemeier, M.; Kay, N. E. *Clin. Diagn. Lab. Immunol.*, **1994**, *1*, 365-72.

[41] Winter, G.; Milstein, C.*Nature*, **1991**, *349*, 293-9.

[42] Morrison, S. L.; Johnson, M. J.; Herzenberg, L. A.; Oi, V. T. *Proc. Natl. Acad. Sci. U. S. A.*, **1984**, *81*, 6851-5.

[43] Boulianne, G. L.; Hozumi, N.; Shulman, M. J. *Nature*, **1984**, *312*, 643-6.

[44] Hu, W. G.; Alvi, A. Z.; Chau, D.; Coles, J. E.; Fulton, R. E.; Suresh, M. R.; Nagata, L. P. *J. Biochem. (Tokyo)*, **2003**, *133*, 59-66.

[45] Bell, S. J.; Kamm, M. A. *Aliment. Pharmacol. Ther.*, **2000**, *14*, 501-14.

[46] Bruggemann, M.; Winter, G.; Waldmann, H.; Neuberger, M. S. *J. Exp. Med.*, **1989**, *170*, 2153-7.

[47] Jones, P. T.; Dear, P. H.; Foote, J.; Neuberger, M. S.; Winter, G. *Nature*, **1986**, *321*, 522-5.

[48] Verhoeyen, M.; Milstein, C.; Winter, G. *Science*, **1988**, *239*, 1534-6.

[49] Hu, W. G.; Chau, D.; Wu, J.; Jager, S.; Nagata, L. P. *Vaccine*, **2007**, *25*, 3210-14.

[50] White, C. A.; Weaver, R. L.; Grillo-Lopez, A. J. *Annu. Rev. Med.*, **2001**, *52*, 125-45.

[51] Hwang, W. Y.; Foote, J. *Methods*, **2005**, *36*, 3-10.

[52] Lorenz, H. M. *Curr. Opin. Mol. Ther.*, **2002**, *4*, 185-90.

[53] Green, L. L. *J. Immunol. Methods*, **1999**, *231*, 11-23.

[54] Leong, S. R.; DeForge, L.; Presta, L.; Gonzalez, T.; Fan, A.; Reichert, M.; Chuntharapai, A.; Kim, K. J.; Tumas, D. B.; Lee, W. P.; Gribling, P.; Snedecor, B.; Chen, H.; Hsei, V.; Schoenhoff, M.; Hale, V.; Deveney, J.; Koumenis, I.; Shahrokh, Z.; McKay, P.; Galan, W.; Wagner, B.; Narindray, D.; Hebert, C.; Zapata, G. *Cytokine*, **2001**, *16*, 106-19.

[55] Choy, E. H.; Hazleman, B.; Smith, M.; Moss, K.; Lisi, L.; Scott, D. G.; Patel, J.; Sopwith, M.; Isenberg, D. A. *Rheumatology (Oxford)*, **2002**, *41*, 1133-7.

[56] Baggiolini, M. *Nature*, **1998**, *392*, 565-8.

[57] Gonzalez-Amaro, R.; Sanchez-Madrid, F. *Crit. Rev. Immunol.*, **1999**, *19*, 389-429.

[58] Rojas, A. I.; Ahmed, A. R. *Crit. Rev. Oral. Biol. Med.*, **1999**, *10*, 337-58.

[59] Sperandio, M. *FEBS J.*, **2006**, *273*, 4377-89.

[60] Gerbitz, A.; Ewing, P.; Olkiewicz, K.; Willmarth, N. E.; Williams, D.; Hildebrandt, G.; Wilke, A.; Liu, C.; Eissner, G.; Andreesen, R.; Holler, E.; Guo, R; Ward, P. A.; Cooke, K. R. *Transplantation*, **2005**, *79*, 536-42.

[61] Saville, L. R.; Pospisil, C. H.; Mawhinney, L. A.; Bao, F.; Simedrea, F. C.; Peters, A. A.; O'Connell, P. J.; Weaver, L. C.; Dekaban, G. A. *J. Neuroimmunol.*, **2004**, *156*, 42-57.

[62] Hynes, R. O. *Trends Cell Biol.*, **1999**, *9*, M33-7.

[63] Joseph-Silverstein, J.; Silverstein, R. L. *Cancer Invest.*, **1998**, *16*, 176-82.

[64] Sorkness, R. L.; Mehta, H.; Kaplan, M. R.; Miyasaka, M.; Hefle, S. L.; Lemanske, R. F. Jr. *Pediatr. Res.*, **2000**, *47*, 819-24.

[65] Salmi, M.; Kalimo, K.; Jalkanen, S. *J. Exp. Med.*, **1993**, *178*, 2255-60.

[66] Kirton, C. M.; Laukkanen, M. L.; Nieminen, A.; Merinen, M.; Stolen, C. M.; Armour, K.; Smith, D. J.; Salmi, M.; Jalkanen, S.; Clark, M. R. *Eur. J. Immunol.*, **2005**, *35*, 3119-30.

[67] Arenzana-Seisdedos, F.; Teyton, L.; Virelizier, J. L. *Scand J.Rheumatol. Suppl.*, **1987**, *66*, 13-7.

[68] Murch, S. H.; Braegger, C. P.; Walker-Smith, J. A.; MacDonald, T. T. *Gut*, **1993**, *34*, 1705-9.

[69] Peper, R. L.; Van Campen, H. *Microb. Pathog.*, **1995**, *19*, 175-83.

[70] Mouser, J. F.; Hyams, J. S. *Clin. Ther.*, **1999**, *21*, 932-42; discussion 31.

[71] Rankin, E. C.; Choy, E. H.; Kassimos, D.; Kingsley, G. H.; Sopwith, A. M.; Isenberg, D. A.; Panayi, G. S. *Br. J. Rheumatol.*, **1999**, *34*, 334-42.

[72] Szulc, B.; Szalaty, H. *Arch. Immunol. Ther. Exp. (Warsz)*, **1980**, *28*, 93-7.

[73] Trinchieri, G.; Perussia, B. *Immunol. Today*, **1985**, *6*, 131-36.

[74] Baumgarth, N.; Kelso, A. *J. Virol.*, **1996**, *70*, 4411-8.

[75] Dumont, F. J. *Curr. Opin. Investig. Drugs*, **2005**, *6*, 537-44.

[76] Hommes, D. W.; Mikhajlova, T. L.; Stoinov, S.; Stimac, D.; Vucelic, B.; Lonovics, J.; Zakuciova, M.; D'Haens, G.; Van Assche, G.; Ba, S.; Lee, S.; Pearce, T. *Gut*, **2006**, *55*, 1131-7.

[77] Beeh, K. M.; Kornmann, O.; Buhl, R.; Culpitt, S. V.; Giembycz, M. A.; Barnes, P. J. *Chest*, **2003**, *123*, 1240-7.

[78] Bischoff, S. C.; Krieger, M.; Brunner, T.; Rot, A.; von Tscharner, V.; Baggiolini, M.; Dahinden, C. A. *Eur. J. Immunol.*, **2003**, *23*, 761-7.

[79] Alam, R.; Forsythe, P.; Stafford, S.; Heinrich, J.; Bravo, R.; Proost, P.; Van Damme, J. *J. Immunol.*, **1994**, *153*, 3155-9.

[80] Conti, P.; Boucher, W.; Letourneau, R.; Feliciani, C.; Reale, M.; Barbacane, R. C.; Vlagopoulos, P.; Bruneau, G.; Thibault, J.; Theoharides, T. C. *Immunology*, **1995**, *86*, 434-40.

[81] Proost, P.; Wuyts, A.; Van Damme, J. *J. Leukoc. Biol.*, **1996**, *59*, 67-74.

[82] Gonzalo, J. A.; Lloyd, C. M.; Wen, D.; Albar, J. P.; Wells, T. N.; Proudfoot, A.; Martinez, A. C.; Dorf, M.; Bjerke, T.; Coyle, A. J.; Gutierrez-Ramos, J. C. *J. Exp. Med.*, **1998**, *188*, 157-67.

[83] Campbell, E. M.; Charo, I. F.; Kunkel, S. L.; Strieter, R. M.; Boring, L.; Gosling, J.; Lukacs, N. W. *J. Immunol.*, **1999**, *163*, 2160-7.

[84] Tekkanat, K. K.; Maassab, H.; Miller, A.; Berlin, A. A.; Kunkel, S. L.; Lukacs, N. W. *Eur. J. Immunol.*, **2002**, *32*, 3276-84.
[85] Belperio, J. A.; Burdick, M. D.; Keane, M. P.; Xue, Y. Y.; Lynch, J. P.; 3rd, Daugherty, B. L.; Kunkel, S. L.; Strieter, R. M. *J. Immunol.*, **2000**, *165*, 461-72.
[86] Gershenwald, J. E.; Fong, Y. M.; Fahey, T. J.; 3rd, Calvano, S. E.; Chizzonite, R.; Kilian, P. L.; Lowry, S. F.; Moldawer, L. L. *Proc. Natl. Acad. Sci. U. S. A.*, **1990**, *87*, 4966-70.
[87] Nishimoto, N.; Hashimoto, J.; Miyasaka, N.; Yamamoto, K.; Kawai, S.; Takeuchi, T.; Murata, N.; van der Heijde, D.; Kishimoto.T. **2005**
[88] Luster, A. D. *N. Engl. J. Med.*, **1998**, *338*, 436-45.
[89] Takeda, K.; Kaisho, T.; Akira, S. *Annu. Re.v Immunol.*, **2003**, *21*, 335-76.
[90] Lee, S. J.; Lee, S. *Curr. Drug Targets Inflamm. Allergy*, **2002**, *1*, 181-91.
[91] Takeda, K.; Akira, S. *Genes Cells*, **2001**, *6*, 733-42.
[92] Boehme, K. W.; Compton, T. *J. Virol.*, **2004**, *78*, 7867-73.
[93] Diebold, S. S.; Kaisho, T.; Hemmi, H.; Akira, S.; Reis e Sousa, C. *Science*, **2004**, *303*, 1529-31.
[94] Guillot, L.; Le Goffic, R.; Bloch, S.; Escriou, N.; Akira, S.; Chignard, M.; Si-Tahar, M. *J. Biol. Chem.*, **2005**, *280*, 5571-80.
[95] Le Goffic, R.; Balloy, V.; Lagranderie, M.; Alexopoulou, L.; Escriou, N.; Flavell, R.; Chignard, M.; Si-Tahar, M. *PLoS Pathog.*, **2006**, *2*, e53.
[96] Termeer, C.; Benedix, F.; Sleeman, J.; Fieber, C.; Voith, U.; Ahrens, T.; Miyake, K.; Freudenberg, M.; Galanos, C.; Simon, J. C. *J. Exp. Med.*, **2002**, *195*, 99-111.
[97] Oude Nijhuis, C. S.; Vellenga, E.; Daenen, S. M.; Kamps, W. A.; De Bont, E. S. *Clin. Diagn. Lab. Immunol.*, **2003**, *10*, 558-63.
[98] Takenaka, R.; Yokota, K.; Ayada, K.; Mizuno, M.; Zhao, Y.; Fujinami, Y.; Lin, S. N.; Toyokawa, T.; Okada, H.; Shiratori, Y.; Oguma, K. *Microbiology*, **2004**, *150*, 3913-22.
[99] Kyburz, D.; Rethage, J.; Seibl, R.; Lauener, R.; Gay, R. E.; Carson, D. A.; Gay, S. *Arthritis. Rheum.*, **2003**, *48*, 42-50.
[100] Schwarze, S. R.; Ho, A.; Vocero-Akbani, A.; Dowdy, S. F. *Science*, **1999**, *285*, 69-72.
[101] Rojas, M.; Donahue, J. P.; Tan, Z.; Lin, Y. Z. *Nat. Biotechnol.*, **1998**, *16*, 70-5.

Received: December 30, 2007 **Revised: January 31, 2008** **Accepted: February 02, 2008**