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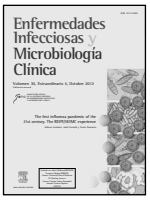

Immunopathogenesis of 2009 pandemic influenza

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A B S T R A C T

Three years after the pandemic, major advances have been made in our understanding of the innate and adaptive immune responses to the influenza A(H1N1)pdm09 virus and those responses' contribution to the immunopathology associated with this infection. Severe disease is characterized by early secretion of pro-inflammatory and immunomodulatory cytokines. This cytokine secretion persisted in patients with severe viral pneumonia and was directly associated with the degree of viral replication in the respiratory tract. Cytokines play important roles in the antiviral defense, but persistent hypercytokinemia may cause inflammatory tissue damage and participate in the genesis of the respiratory failure observed in these patients. An absence of pre-existing protective antibodies was the rule for both mild and severe cases. A role for pathogenic immunocomplexes has been proposed for this disease. Defective T cell responses characterize severe cases of infection caused by the influenza A(H1N1)pdm09 virus. Immune alterations associated with accompanying conditions such as obesity, pregnancy or chronic obstructive pulmonary disease may interfere with the normal development of the specific response to the virus. The role of host immunogenetic factors associated with disease severity is also discussed in this review. In conclusion, currently available information suggests a complex immunological dysfunction/alteration that characterizes the severe cases of 2009 pandemic influenza. The potential benefits of prophylactic / therapeutic interventions aimed at preventing/correcting such dysfunction warrant investigation.

Inmunopatogénesis de la gripe pandémica de 2009

Palabras clave:
Gripe
H1N1pdm
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Adaptativa
Hipercitoquinemia
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Células T
Anticuerpo
Disfunción inmunológica
Escape viral

R E S U M E N

El posterior estudio de la gripe pándemica de 2009 hasta la actualidad ha dado como fruto un mayor conocimiento de las respuestas inmunológicas innatas y adaptativas al virus de la gripe A(H1N1)pdm09 y de la contribución de las respuestas a la inmunopatología asociada con esta infección. Se ha comprobado que la enfermedad grave se caracteriza por la secreción de citocinas proinflamatorias e inmunomoduladoras desde el principio de la enfermedad. En los pacientes con neumonía viral grave la secreción de citocinas se mantuvo y estaba relacionada directamente con el grado de replicación viral en el tracto respiratorio. Si bien las citocinas tienen un papel importante en la defensa antiviral, sin embargo la persistencia de hipercitoquinemia puede dañar el tejido inflamatorio y ser uno de los motivos que provocan el fracaso respiratorio observado en estos pacientes. En casi todos los casos leves y graves de enfermedad se observó ausencia de anticuerpos protectores preexistentes. Se ha propuesto que los inmunocomplejos patogénicos tienen un papel en esta enfermedad. Los casos graves de infección causados por el virus de la gripe A(H1N1)pdm09 se caracterizan por una respuesta defectuosa de las células T. En el desarrollo normal de la respuesta específica al virus pueden interferir trastornos inmunológicos asociados con situaciones concomitantes como obesidad, embarazo o enfermedad pulmonar obstructiva crónica. Los autores de esta revisión plantean también el papel que tienen los factores inmunogenéticos del huésped en la gravedad de la enfermedad. La gripe pandémica de 2009, de acuerdo con la información disponible 3 años más tarde, se caracteriza por una disfunción/alteración inmunológica compleja, por lo que los potenciales beneficios de las intervenciones terapéuticas deben ir dirigidos a corregir dicha disfunción y siempre garantizados mediante la investigación.

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Introduction

The appearance of the first pandemic of the twenty-first century, caused by a new variant of the H1N1 influenza virus—influenza A(H1N1)pdm09—has represented a challenge for health systems and the scientific community, revealing the dramatic importance of effective approaches to the prevention of viral infection.

The majority of influenza A(H1N1)pdm09 infections are mild and self-limiting in nature,[1-3] but a small percentage of patients require hospitalization and specialized attention in intensive care units (ICUs). Although the mortality rate from seasonal influenza is generally low and tends to occur in the extreme ages of life (children and the elderly), clinical and epidemiological studies showed that in the 2009 pandemic the vast majority of severe cases occurred in young adults.[4-8] The importance of accompanying co-morbidities as risk factors for severe disease following infection by the influenza A(H1N1)pdm09 virus has been confirmed in many studies.[5,6,8-10]

Three years later, major advances have been made in our understanding of the innate and adaptive immune responses to the influenza A(H1N1)pdm09 virus. Host responses to the virus and their contribution to immunopathology have been assessed using animal and cellular models. In addition, an increasing number of studies have described immune responses associated with severe human disease. These studies have revealed the complex balance between immune protection and immune-mediated damage in this disease.

The immune system is designed to protect and maintain homeostasis and the ability of an organism to adapt to the environment.[11] Therefore, it plays a key role in viral clearance. Infection of the respiratory tract causes the activation of a non-specific TLR-mediated innate immune response, which is our first line of defense against invading viruses. Later in the course of the infection, the adaptive immune response would specifically eliminate the virus, generating long-term immune memory against the virus.[12]

However, there is evidence of the potential role of host immune responses as contributors to tissue damage and to the respiratory failure observed in the most severe cases caused by influenza infection. We and other authors have previously identified specific host immune response signatures in severe and mild severe acute respiratory syndrome CoV, H5N1 and respiratory syncytial virus infections.[13-17] In these studies, severe disease and fatalities were characterized by early hyper-expression of the chemokines and cytokines involved in anti-viral responses.

This article will review the currently available knowledge on the immunopathogenesis of influenza A(H1N1)pdm09.

Hypercytokinemia as a host response signature in severe pandemic influenza

Early hypercytokinemia as a hallmark of severe disease

At the beginning of the 2009 pandemic, the absence of available information on the role played by the host immune response in viral clearance and immunopathogenesis resulted in an urgent need for studies addressing these issues.

Information from in vitro cellular models was the first to arrive, and they initially reported that the influenza A(H1N1)pdm09 virus (compared to H5N1 and seasonal influenza strains) was a poor inducer of cytokines in human macrophages, dendritic cells and respiratory epithelial cells.[18,19] In contrast, evidence coming from later clinical studies on patients infected with the pandemic virus pointed out the limitations of cellular models. A first report published in December 2009 revealed that severe disease caused by the influenza A(H1N1)pdm09 virus was characterized by the presence of high systemic levels of cytokines, chemokines and other immune mediators from the early stages of the disease.[20] Further studies confirmed this finding,[21-23] identifying hypercytokinemia as a host response signature in severe pandemic influenza (Fig. 1).

Infection by the influenza A(H1N1)pdm09 virus induced the secretion of antiviral defense-related chemokines (IP-10, MIP-1β, MCP-1 and IL-8). These chemotactic molecules mobilize T lymphocytes, monocytes, macrophages and neutrophils to the site of infection[24] to fight the infection.[25] However, an accumulation of these cells may contribute to inflammatory-mediated damage to the infected tissue. Infected patients also exhibited elevated levels of other pro-inflammatory immune mediators that stimulate T helper 1 (Th1)— interferon (IFN) gamma (IFN-δ), tumour necrosis factor (TNF-α), interleukin (IL) 15, IL-12p70—. Th1 adaptive immunity plays a central defensive role against intracellular microbes such as viruses,[26] and it may be necessary for influenza virus clearance during infection.[27] On the other hand, Th1 cytokines may, as chemokines, contribute to tissue injury. Studies on cytokine profiles also revealed elevation of 2 Th17 related cytokines (IL-9, IL-6) in the early course of the severe cases of pneumonia caused by H1N1pdm. Th17 immunity has important functions in anti-bacterial and anti-viral defense, but it is also involved in the pathogenesis of several autoimmune/allergic diseases such as systemic lupus erythematosus and asthma.[28-30] However, a beneficial role of IL-17 in lethal influenza has been previously proposed.[20,21] Additionally, granulocyte colony-stimulating factors (G-CSF) —which has been described as interfering with the synthesis of IL-17[31]—has been reported to be directly associated with the risk of death in critically ill patients. On the contrary, IL-17 was described as a protective factor against mortality.[32] These results thus support the imbalanced pro- and anti-Th17 responses in severe pandemic influenza, highlighting the importance of the regulation of the Th17 axis in this disease. Regarding IL-6, there is a fairly broad consensus in the literature on this cytokine as a potential biomarker for severe influenza A(H1N1)pdm09 infection, in both human and in mouse studies.[20-23,33,34] Elevated systemic levels of IL-6 were strongly associated with ICU admission and with fatal outcomes. IL-6 has been described as present in the bronchoalveolar lavage of severe patients.[35] In mice, infection with influenza A(H1N1) pdm09 consistently triggered severe disease and increased IL-6 levels in both the lungs and in serum. Furthermore, in animal and clinical studies, global gene expression analysis indicated a pronounced IL-6-associated inflammatory response.[34-36]

IL-6 is a pleiotropic cytokine significantly implicated in various facets of the immune response. IL-6 has been implicated in the cytokine storm following avian influenza A H5N1, and in severe acute respiratory syndrome infection.[17,37,38] IL-6 levels have been associated with symptom duration and severity in cases of seasonal influenza infection.[39-41] The available information thus seems to point towards a detrimental role of high levels of this mediator in the pathogenesis of infection with the pandemic virus. However, a knockout mouse model for IL-6[34] has recently demonstrated that the absence of IL-6 had no significant major clinical repercussions, suggesting that IL-6 does not play an essential non-redundant role in influenza A(H1N1) pdm09 infection in mice. An absence of data on animal models over-expressing IL-6 in response to influenza infection precludes definitive conclusions on the pathogenic role of IL-6 in this disease.

Severe cases showed early increases of vascular endothelial growth factor (VEGF) levels in the blood. This is a potent angiogenic factor, which is stimulated in hypoxic patients,[42] and is thought to increase permeability of the bronchial airway epithelium following infection by respiratory viruses.[43]

Cases of severe pandemic influenza disease were also marked by high levels of 2 immunomodulatory cytokines (IL-10 and IL-1ra). The secretion of these molecules may represent an attempt to prevent prolonged inflammatory damage promoted by the release of cytokines and chemokines, or alternatively may be a viral mechanism of evasion from the host immune response.[44-46]

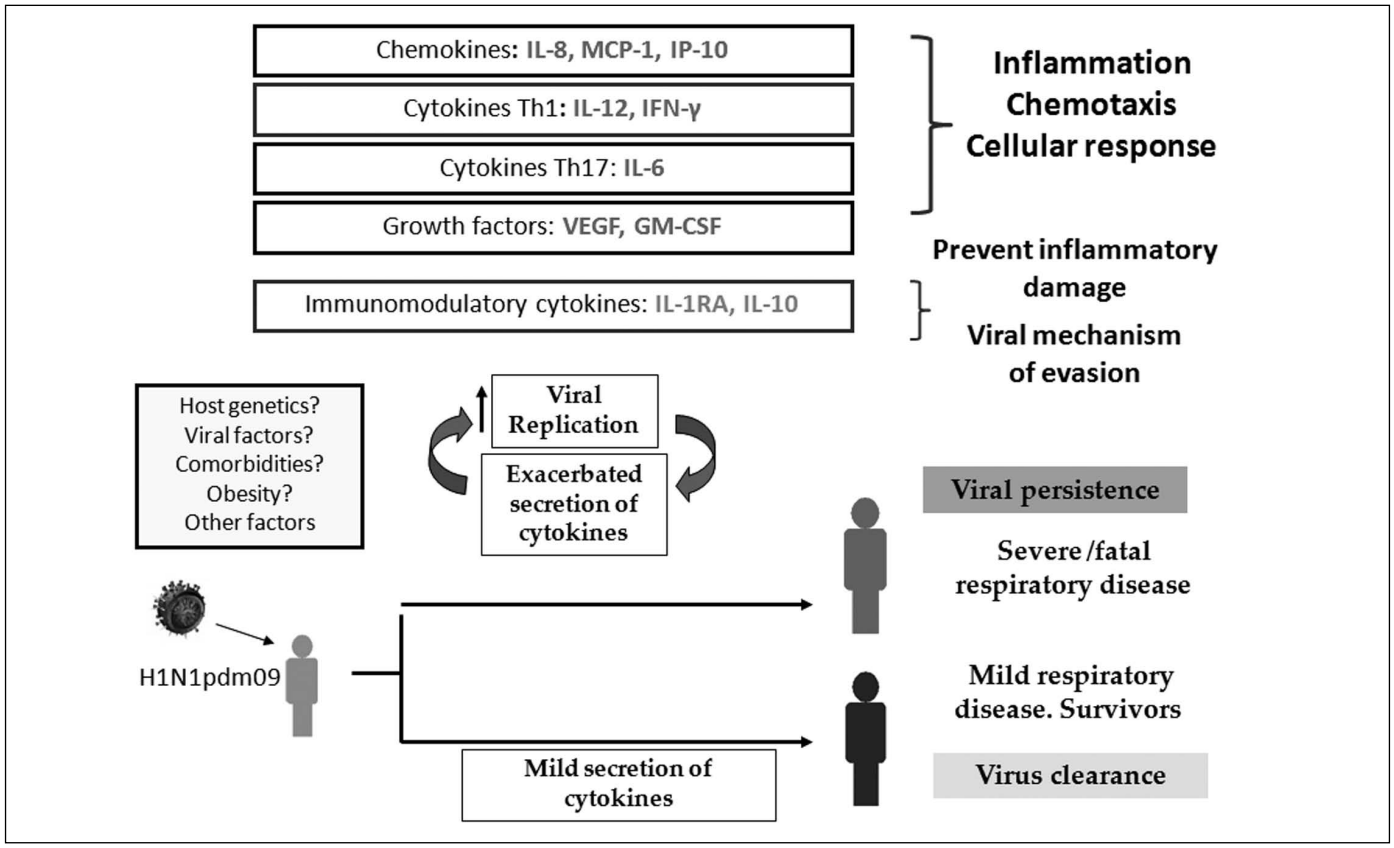

Figure 1. Hypercytokinemia as a host response signature in severe pandemic influenza. Based on results from Bermejo-Martín et al.[20,36] and Almansa et al.[51]

Association between pharyngeal viral secretion and the host cytokine response

Severe pandemic influenza disease was characterized by poor control of the virus from the early moments of the infection, in the absence of previous protective antibodies.[20,36] Moreover, numerous studies have found that severe patients were unable to reduce viral load until the very late stages of the disease, despite antiviral treatment,[21,47,48] suggesting poor control of the virus from the onset of infection. This idea is reinforced by the observed association between the persistence of virus excretion and the pathology and severity of these patients.[49,50] In turn, as occurs in fatal cases of infection by influenza A/H5N1 virus,[17] levels of the virus secreted by the respiratory tract are directly associated with cytokine levels in the blood from the beginning of the disease,[51] reinforcing the notion of an immune dysfunction in severe pandemic influenza.

Persistent hypercytokinemia in severe disease

Hypercytokinemia persisted in the most severe cases, which could have perpetuated the inflammatory damage and, in consequence, the respiratory failure observed in these patients.[21,36] Similarly, other clinical studies demonstrated that high plasma levels of IL-6, IL-8 and MCP-1 correlated with the extent and progression of pneumonia.[49] The most severe cases also showed persistent viral shedding, again indicating poor control of viral replication.

The role of antibodies

Cross-immunity and antibody production

One of the most striking features of the 2009 pandemic was the low proportion of elderly individuals infected by the new virus, compared to seasonal influenza.[4-8] In addition, severe illness caused by the new variant predominated in young patients, with 90% of deaths occurring in patients <65 year old,[33,52] which is contrary to the normal trend in seasonal influenza.

It is believed that adults born after 1956 (i.e., over 60 years old) have suffered previous exposures to antigenically related influenza viruses, developing in consequence cross-reactive antibodies with the ability to recognize the 2009 strain.[53,54] Studies on antibody prevalence show the presence of cross-reacting antibodies in as much as in 33% of the over-60 population.[55] In addition, other studies reveal the molecular basis for such cross-reactivity between the 1918 and the 2009 pandemic viruses. In these articles, the authors suggest that antibodies against the 1918 virus recognize both hemagglutinins from the 1918 and from the 2009 pandemic viruses.[56-58]

This result is consistent with the fact that young adults admitted to the ICU during the 2009 pandemic lacked protective antibodies in the early stages of the disease, as revealed by hemagglutination inhibition (HAI) and micro-neutralization assays.[20,36] However, the absence of early HAI and neutralization activity was the rule in young patients, independent of disease severity and outcome. It is important to note that most of these critical patients were able to mount specific antibody responses against the pandemic virus, regardless of severity.[36] This suggests that factors other than the development of specific antibodies contribute to the pathogenesis of severe pandemic influenza.

Pathogenic immunocomplexes

Severe disease in middle-aged adults has been explained on the basis of the pre-existence of cross-reactive antibodies with low affinity for influenza A(H1N1)pdm09 that would not be able to block the virus but instead would form pathogenic immunocomplexes,[59] which would accumulate in the lung, triggering inflammation-

mediated damage. These antibodies would not be present yet in children, who would not develop immunocomplexes and in consequence would not develop severe disease.

Cellular immune responses

Cross-reactive T CD4+ lymphocytes (Th lymphocytes) and T CD8+ cytotoxic lymphocytes (CTLs) established by vaccination campaigns or natural infection by the seasonal influenza A virus have been reported to contribute to clearance of the H1N1pdm virus from the lungs.[60,61] Even in the absence of protective antibody responses, individuals vaccinated against seasonal influenza A may still benefit from pre-existing cross-reactive memory CD4 T cells thus reducing their susceptibility to the influenza A(H1N1)pdm09 virus.[60] T CD4 effector cells are essential for virus clearance, but in turn may contribute to the hypercytokinemia observed in the most severe cases caused by influenza A(H1N1)pdm09 infection. In fact, a study on a murine model demonstrated that depletion of T cells prevented immunopathology, although with decreased viral clearance.[62]

In turn, CD8+ T cells are known to release cytotoxic molecules (granzyme and perforin) and antiviral cytokines (TNF-α and IFN-δ), which are essential for mediating the elimination of infected cells. However, various studies indicate that releasing these molecules may also contribute to the mediation of lung pathology. For example, mice deficient in Egr-1 (epithelial early growth response 1) did not develop lung injury, suggesting a role for ERK kinases induced Egr-1 in the immunopathology mediated by CTLs.[63] In addition, experimentally infected TLR3 K.O mice produced lower levels of inflammatory mediators (RANTES, IL-6, IL-12), showed milder lung infiltration by CTLs, and increased survival rate.[64]

Similarly, a small report on human autopsy tissues documented diffuse alveolar damage, hemorrhage and necrotizing bronchiolitis in the lungs of patients who died from influenza A(H1N1)pdm09 infection. Immunohistological examination revealed an aberrant immune response associated with marked expression of TLR-3 and IFN-δ and a large number of CD8+ T cells and granzyme B+ cells within the lung tissue,[65] highlighting the role of cellular immune responses in the immunopathology of H1N1pdm influenza infection.

T cells from influenza A(H1N1)pdm09-infected patients presenting with a severe clinical course have been described as resulting in impaired effector cell differentiation and as failing to respond to mitogenic stimulation.[66] In addition, T cell anergy is observed during the severe acute phase of the infection.[66] The adaptive immune response of influenza A(H1N1)pdm09-infected patients has been reported to be characterized by decreases of CD4- lymphocytes and of B-lymphocytes and by increases in T-regulatory lymphocytes.[67] The latter cells may suppress the development of specific responses against the virus.

In addition, critical pandemic influenza illnesses coursed with lower expression in the white blood cells (WBC) of a group of genes key to the development of antigen presentation and adaptive immune response.[36] This finding may reflect a state of peripheral immune-deficiency in severe pandemic influenza, or alternatively respond to re-distribution of WBC to the lungs. In any case, hypo-expression of human leukocyte antigen (HLA) and T-cell-related genes in peripheral blood constitutes a molecular signature of severe pandemic influenza, which may help to identify patients at risk of developing complicated disease.

Deficiencies in the cellular immunity occurring in severe cases of influenza A(H1N1)pdm09 infection could help explain the poor control of the virus observed in these patients, and the increased risk of these patients to suffer from bacterial over-infections.

Patients with previous immunological disorders

Chronic obstructive pulmonary disease (COPD), asthma, cardiovascular disease, hypertension and diabetes have been reported as risk factors for critical illness following infection with the pandemic virus.[5,6,8-10] In addition, during the 2009 pandemic, pregnancy[68-70] obesity,[71,72] and smoking[10] were identified as risk factors for severity. Chronic respiratory diseases such as COPD[73] and asthma are characterized by a basal predisposition to the release of inflammatory mediators. Results with cell cultures and mouse models have previously demonstrated that a high level of cytokines could itself prevent the development of and appropriated immune response against viruses, affecting dendritic cell function along with HLA-II mediated antigen presentation.[23,66,74] In a mouse model of COPD, animals infected with influenza virus showed an exacerbated inflammatory response to infection.[75]

In addition to the risks associated with obesity, such as cardiovascular disease or diabetes, immunological alterations in the obese may contribute to its role as a risk factor in the 2009 influenza pandemic.[76] Adipocytes have structural similarities with the immune cells and perform certain functions related to them, such as the release of inflammatory mediators. Furthermore, differentiation of macrophages in the adipose tissue is conditioned by the metabolic environment and immune cells in turn are able to control lipid and glucose metabolism, suggesting an immune metabolic axis. A chronic caloric excess could interfere with the mechanisms of the immune response.[74] Obese mice infected with the 2009 pandemic virus exhibited significant higher morbidity and mortality compared to non-obese mice.[77]

During pregnancy, a number of changes are induced in the immune system of the mother, aimed at tolerating the fetus. Although these changes in the immune system are not fully understood, it is believed that they may increase the severity of some infections.[78,79] In pregnant women, the balance between pro- and anti-inflammatory factors seems to be key.[79] In addition, changes in the peripheral levels of immune mediators such as IFNδ, TNF, VEGF, G-CSF, eotaxin, and MCP-1 may impact the proper performance of the immune response.[80] The increased mortality detected in pregnant female mice infected with the influenza A(H1N1)pdm09 virus is associated with increased infiltration of neutrophils and macrophages in the lungs of these animals. In addition, pregnant mice showed higher levels of chemokines and pro-inflammatory cytokines, lower respiratory epithelial regeneration and poorer fetal development than non-pregnant mice.[81,82]

Therefore, basal immune alterations and/or the presence of a previous pro-inflammatory state favored by the presence of conditions such as COPD, obesity and pregnancy may impact the normal development of specific immune responses against the H1N1pdm virus, increasing the risk of developing severe forms of the infection.

Immunogenetic factors associated with severe respiratory illness

Although few studies have systematically evaluated the influence of genetic polymorphisms on susceptibility to H1N1pdm infection, the available data suggest that host immunogenetic variations may play an important role in the final outcome following infection by the virus.[83]

In a recent review,[83] the authors propose that the potential immune dysfunction caused by underlying genetic polymorphisms may lead to impaired responses and would therefore be associated with adverse outcomes (Table 1).

Immunological based therapies

Steroids and macrolides

An issue that has generated discussion in the context of pandemic influenza is the use of steroids and macrolides for the treatment of

Table 1
Genetic polymorphisms of interest in influenza A(H1N1)pdm09 susceptibility and severity

Gene	Polymorphism	Significance
CCR5	CCR5 Δ 32	Increased allele frequency among Canadian H1N1 ICU cases[83]
FcδRIIa, IGHG2	IGHG2 *n/*-n, FcδRIIa-R131H	Polymorphisms previously linked to IgG2 deficiency, but not corroborated in influenza A(H1N1)pdm09 patients[20,84,85]
NLRP3	2107C/A (Q705 K), rs4612666 (intron 7), rs10754558 (3' UTR)	Association with dysregulation of inflammatory response (2107), alteration of NLRP3 mRNA stability and enhancer activity[86,87]
HLA	Various alleles	Influenza-specific CTLs responses exhibit varying frequency and magnitude across various HLA alleles[88]

CTLs: cytotoxic lymphocytes; HLA: human leukocyte antigen; ICU: intensive care unit.
Adapted from Juno et al.[82]

complicated cases. The use of steroids has been related to increased risks of bacterial co-infection,[84,85] while its use has not been associated with significant clinical benefit in patients with pneumonia caused by the pandemic virus.[86] It has been previously reported that, in conditions characterized by high oxidative stress or by the presence of high levels of pro-inflammatory cytokines, steroids are no longer active.[87,88] Therefore the role of steroids in the treatment of disease caused by the pandemic virus is not currently recommended.

Macrolides have received considerable attention for their anti-inflammatory and immunomodulatory actions beyond the antibacterial effect, but their benefit is still uncertain in a wide spectrum of respiratory viral infections. It has been shown that macrolides reduce viral titers and down-modulate the secretion of cytokines secondary to infection by rhinovirus, respiratory syncytial virus and influenza.[89] Thus, it has been postulated that reductions in pro-inflammatory cytokines may modulate influenza-virus-induced inflammation and the severity of the disease.[90] In pediatric patients suffering from influenza infection, the addition of clarithromycin to oseltamivir augments the production of secretory immunoglobulin A (sIgA) and restores local sIgA levels, enhancing the mucosal immune response in these children.[91]

Treatment with hyperimmune serum

In a clinical assay including patients with severe H1N1pdm infection requiring intensive care, treatment of the infection with convalescent plasma reduced respiratory tract viral load, serum cytokine response, and mortality, providing evidence for the protective effect of hyperimmune serum in the treatment of this disease.[92]

Hemoperfusion for reduce hypercytokinemia

Another possible treatment for severe cases caused by influenza viruses is hemoperfusion. Hemoperfusion using polymyxin B-immobilized fiber column (PMX) has been shown in a case report to reduce levels of inflammatory mediators in plasma, improving oxygenation.[93] In particular, PMX hemoperfusion reduced the high-morbidity group box 1 levels, which was described as a therapeutic target in an animal model of severe influenza.[94]

The use of immunomodulatory drugs opens major avenues to develop therapies in combination with antivirals for the treatment of influenza infection.[95] This new kind of treatment, based on reducing

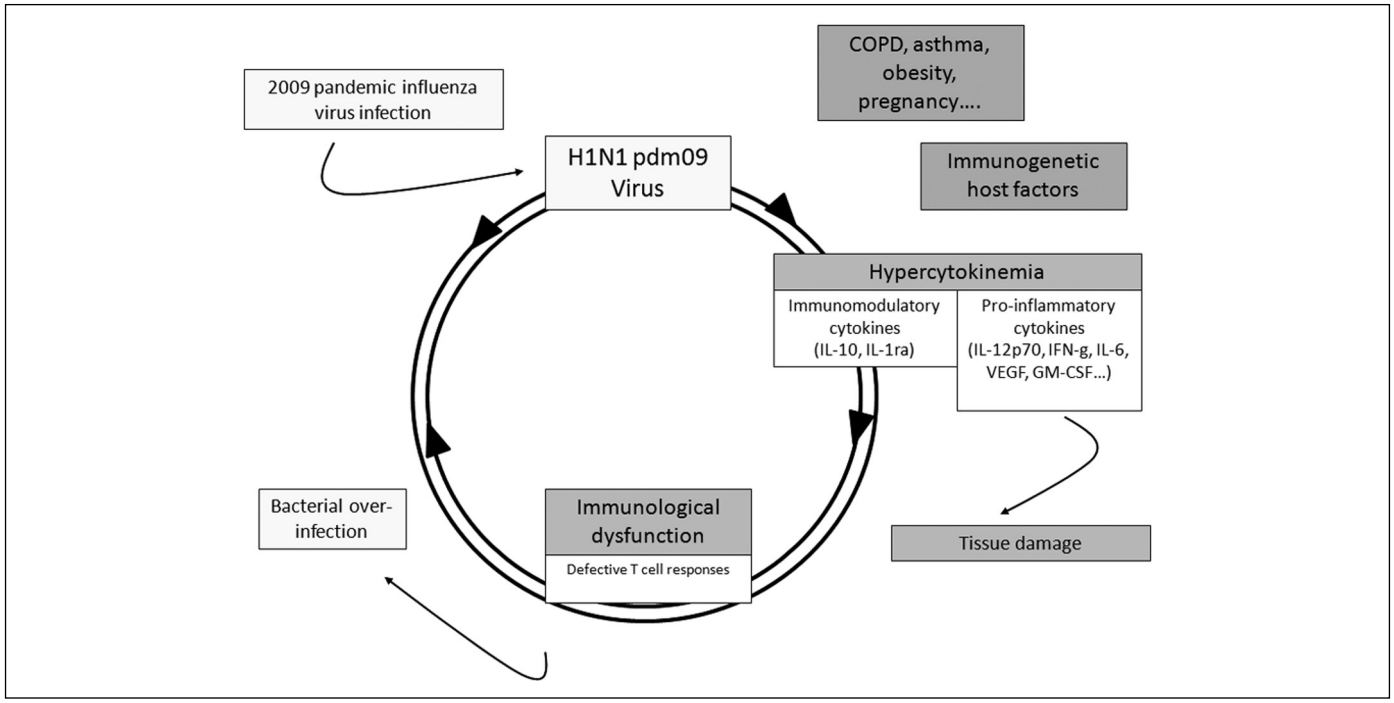

Figure 2. Un-virtuous circle of the immune response in severe influenza A(H1N1)pdm09 infection. Modified from Bermejo-Martín et al.[36]

the inflammatory mediators, highlights the important role of hypercytokinemia in the pathology of the disease, and encourages further research on the use of targeted therapeutic strategies to combat inflammation to resolve the disease caused by the pandemic virus.

The vast majority of patients developing complicated disease during the 2009 pandemic were non-vaccinated individuals with no pre-existing protective antibodies. In the light of the information presented in this review, pandemic influenza vaccination may offer additional benefits by reducing the number of naïve individuals, not only among patients at risk of developing complications but also in the normal population, thus suggesting a wide vaccination coverage approach when facing future pandemics.

Conclusions

The available evidence suggests a deregulated/altered immune response in those patients suffering from the most severe forms of the 2009 pandemic influenza disease. This response was characterized by exacerbated secretion of pro-inflammatory and immunomodulatory cytokines in the early-innate phase of the response to the virus and by deregulated T cell responses during the development of specific adaptive immunity. These alterations translate into poor control of the infection, creating a vicious circle of viral replication – cytokine production to viral escape, which could perpetuate inflammation-mediated tissue damage and respiratory failure (Fig. 2). Individual and environmental factors causing this altered immune response remain to be elucidated.

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Conflicts of interest

All authors declare that they have no conflicts of interest in this article.

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