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A potential role for monoclonal antibodies in prophylactic and therapeutic treatment of influenza

Nicasio Mancini *[,1], Laura Solforosi [1], Nicola Clementi, Donata De Marco, Massimo Clementi, Roberto Burioni

Laboratorio di Microbiologia e Virologia, Università "Vita-Salute" San Raffaele, Milano, Italy

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ABSTRACT

The role of humoral response in the effective control of infection by influenza viruses is well known, but the protection is usually limited to the infecting or vaccinating isolate and to few related strains. Recent studies have evidenced the existence of B-cell epitopes broadly conserved among different influenza subtypes recognized by monoclonal antibodies endowed with unprecedented broad activity. In this review, all major monoclonal antibodies directed against different influenza virus proteins are reported and their potential in the design of new anti-influenza prophylactic or therapeutic strategies is discussed.

1. Introduction

Influenza is the most recurring respiratory affection in humans, and its socio-economical impact has been often dramatic. During the 20th century, influenza A viruses have afflicted the human race with three pandemics: in 1918 caused by a virus belonging to the H1N1 subtype, in 1957 caused by a H2N2 isolate, and in 1968 caused by a H3N2 isolate (Palese, 2004). In 2009 a H1N1 reassortant strain has caused the first pandemic of the new century (Girard et al., 2010) and is now the cause, together with H3N2, of the ongoing seasonal epidemic. The 2009 pandemic proved to be not as severe as initially feared, but it drew attention to the continuous risk of a major influenza pandemic caused by a completely novel strain seriously threatening global public health. This inevitable risk, due to episodic major antigenic changes on the surface of influenza virions (antigenic shift) will always face humankind and the potentially dramatic impact of the next major pandemic is not predictable.

Apart from containment and preventive public health strategies which will not be discussed in this review, the only prophylactic or therapeutic measures now available for governments and public health agencies around the world are two classes of anti-influenza drugs (neuraminidase inhibitors and M2 blockers) and the annual vaccination campaigns. Several concerns have been raised regarding the real cost-effectiveness of the broad prophylactic use of available drugs (Burch et al., 2009a,b), which have been and are currently stockpiled by public health agencies in anticipation of a possible future pandemic. The concerns include the need of their prompt administration to be effective (Beigel and Bray, 2008), the rapid emergence of resistant isolates (Cheng et al., 2009; Ramirez-Gonzalez et al., 2011) and several associated side-effects especially in high-risk categories, such as children and pregnant women (Burioni et al., 2009a,b; Kitching et al., 2009). In addition, current influenza vaccination campaigns are based on a presumptive process: each year, a new vaccine is prepared that aims to match the strains predicted to circulate in the coming flu season. This is because the virus continuously undergoes genetic mutation to escape from the host immune response and the resulting hypervariability is particularly evident on the two major influenza surface proteins, hemagglutinin (HA) and neuraminidase (NA) (Gamblin and Skehel, 2010). This continuous variability (antigenic drift) is the molecular basis causing seasonal influenza infections (Carrat and Flahault, 2007; Webster et al., 1992), and it is the molecular factor determining the already observed mismatches between the predicted vaccinal strains and the circulating strain, therefore causing vaccine ineffectiveness (Monto et al., 2009). Moreover, vaccine ineffectiveness is highly probable in the case of the major antigenic variations associated with a future novel pandemic strain.

In order to overcome the drawbacks of the available prophylactic and therapeutic approaches, new broad-range strategies are therefore needed and, accordingly, several research strategies have been already described (Ansaldi et al., 2009; Monto et al., 2009; Nabel and Fauci, 2010; Stanekova and Vareckova, 2010; Steel et al., 2010). A pivotal role in this field will certainly be played

* Corresponding author. Address: Laboratorio di Microbiologia e Virologia, Università "Vita-Salute" San Raffaele, DIBIT2, 2° piano, via Olgettina 58, 20132 Milano, Italy. Tel.: +39 02 2643 4195; fax: +39 02 2643 2640.
E-mail address: mancini.nicasio@hsr.it (N. Mancini).
[1] These authors contributed equally to the paper.

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by the identification of monoclonal antibodies (mAbs) capable of targeting neutralizing epitope broadly shared among different subtypes (Karlsson Hedestam et al., 2008; Nabel and Fauci, 2010). Indeed, it is known that antibodies play an important role in the natural protection against influenza viruses and that HA is the main target for virus-neutralizing Abs (Epstein et al., 1993; Gamblin and Skehel, 2010; Gerhard et al., 1997; Palladino et al., 1995). In particular, in several animal models secretory anti-HA IgA are important in protecting the upper respiratory tract mucosa in the initial phase of the infection, whereas serum IgG (the Ig isotype mostly elicited after intramuscular administration of the inactivated influenza vaccine) are crucial in preventing lung infection and in limiting its spread to other organs (Epstein and Price, 2010; Renegar et al., 2004). However, although a single influenza infection or vaccination provides lifelong immunity against the homotypic strain and a limited number of antigenically correlated strains, the host remains susceptible to infection with a novel flu variant due to HA hypervariability (Carrat and Flahault, 2007; Webster et al., 1992). Moreover, a possible paradoxical immunological consequence of the protective immunity against a limited panel of strains is that it may reduce the effectiveness of the immune response elicited against other drifted strains. This phenomenon, named "original antigenic sin", suggests that a virus causing a first infection may somehow imprint the immune system to preferentially recognize its own antigenic features; as a consequence, subsequent responses to drifted variants would be mainly limited to antibodies cross-reacting with the old strains and therefore not necessarily neutralizing the new variants (Dormitzer et al., 2011).

Overall, when considering anti-influenza immunity, three main different types of protection may be distinguished:

i. Homologous immunity: that is, immunity against a single influenza isolate. This kind of protection is the one observed after an infection or a vaccination, but it usually confers a very limited protection against other isolates.
ii. Homosubtypic immunity: that is, immunity against isolates belonging to the same subtype. This kind of immunity may be seen after a natural infection or a vaccination, but it is lost when the mutation rate among different strains increases considerably.
iii. Heterosubtypic immunity: that is, immunity against isolates belonging to different subtypes. This kind of immunity is uncommon both after natural infection or vaccination especially against highly divergent subtypes. However, this kind of immunity has to be considered as the potential gold standard for really "broad-range" prophylactic or therapeutic approaches.

Until recently, heterosubtypic immunity was considered possible only after the elicitation of a T-cell immune response against highly conserved influenza inner proteins, such as M1, NP and NS1 proteins (Assarsson et al., 2008; Jameson et al., 1998). Indeed, an important role in the control of viral spread and in the viral clearance from infected tissues is played by cytotoxic T-lymphocytes (CTL) which recognize viral peptides presented via major histocompatibility type-I (MHC-I) on the surface of infected cells. It has been described that CTL depletion in animal models leads to higher viral titers and to a more severe clinical course of the infection (Liang et al., 1994). Other recent studies confirm the beneficial role of CTL response in limiting the severity of influenza infection also in humans (Kreijtz et al., 2008; Tu et al., 2010). More importantly, there is increasing evidence in humans that memory T-cells specific to conserved epitopes on the above-mentioned influenza proteins may protect from viral strains belonging to different subtypes (Kreijtz et al., 2007,2008; Lee et al., 2008; Tan et al., 2010; Tu et al., 2010). On the basis of this data new vaccinal strategies focused on the elicitation of a heterosubtypic protective T-cell response are under study as a possible support to the "classical" approach focused on eliciting neutralizing antibodies (Epstein and Price, 2010).

At the same time, several studies in mouse models suggest that also humoral immunity, especially in its IgG component, when directed against conserved epitopes contributes to heterosubtypic protection (Nguyen et al., 2001; Quan et al., 2008a,b; Takada et al., 2003; Tumpey et al., 2001). As an example, a heterosubtypic immune response was successfully elicited in infected β2-microglobulin knockout mice or in mice lacking the secretory IgA J-chain, evidencing that neither MHC-I-restricted T-cells nor secretory IgA are absolutely required for cross-protection (Epstein et al., 1997). In another study, the role of cross-reactive mucosal IgA has also been described in subjects vaccinated with intranasal inactivated vaccines (Tamura, 2010). Overall, a better comprehension of the role of humoral response in conferring such heterosubtypic anti-influenza immunity and of its interplay with T-cell response is therefore crucial for the development of new anti-influenza strategies. In this perspective, the role of well characterized mAbs may be extremely important.

In this review we will overview all major anti-influenza mAbs described in the literature, with particular attention reserved to those of human origin especially among those directed against HA. In each of the four paragraphs, a brief molecular description of the targets will be followed by comments on the prophylactic and therapeutic potential of available mAbs.

2. Monoclonal antibodies directed against influenza A hemagglutinin (HA)

Hemagglutinin (HA) is the major influenza surface protein, with approximately 500 molecules per virion. Sixteen different types of HAs (H1–H16) have been recognized, but among them only three subtypes (H1, H2 and H3) have been recognized to establish pandemics in the human population (Fouchier et al., 2005). The 16 HA subtypes are further classified on the basis of phylogenetic sequence in two groups, group 1 including, among others, H1, H2 and H5 subtypes, and group 2 including, among others, H3 subtype (Lambert and Fauci, 2010; Nabel and Fauci, 2010).

HA is synthesized as a single polypeptide precursor (HA0) which is cleaved by cellular proteases into two subunits, HA1 or the binding subunit, and HA2 or the fusion subunit (Fig. 1) (Gamblin and Skehel, 2010). The two subunits remain covalently linked to each other through a disulfide bond. On the viral envelope each HA molecule is organized in trimeric spike structures, with the globular HA1 domain mostly exposed on the surface and the HA2 fusogenic domains mainly constituting the fibrous stem of each trimer. The N-terminus of the HA2 domain contains a sequence of about 20 mostly hydrophobic aminoacids, particularly hidden in the HA structure, which constitutes the so-called "fusion peptide" (Gamblin and Skehel, 2010). The pivotal role of HA in the viral cycle and its exposition on the viral envelope make it the primary viral antigen targeted by the host's antibody response and the only antigen inducing a neutralizing antibody response. This is the reason why HA is the most variable influenza protein and why its variations, especially on the highly exposed HA1 subunit, are the main responsible for the immune escape of influenza viruses.

Neutralizing mAbs act by blocking either of the two functions of HA: virus binding to sialic acid on the cell surface or virus fusion with the endosomal membrane (Skehel and Wiley, 2000). Binding–blocking mAbs are typically directed against antigenic sites surrounding the receptor binding pocket in the membrane distal HA1 subunit (Wiley et al., 1981). Antibodies with similar features are the most abundant neutralizing antibodies produced in the