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Broad-range neutralizing anti-influenza A human monoclonal antibodies: new perspectives in therapy and prophylaxis

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SUMMARY

Broadly neutralizing monoclonal antibodies (mAbs) directed against different subtypes of influenza A viruses are novel tools for the potential development of effective anti-influenza prophylactic and therapeutic strategies. In both cases, the main candidates for passive transfer and new vaccine development are represented by protective mAbs directed against influenza hemagglutinin (HA). A large number of mAbs directed against influenza HA has been developed to date. However, even if they can be useful and contribute to develop new vaccinal strategies, only few of them can be a good candidate for human administration. In this review, we will describe the most relevant human mAb directed against influenza HA able to recognize highly divergent influenza isolates and possibly useful for human therapy and prophylaxis.

KEY WORDS: Neutralizing antibody, Monoclonal antibody, Antigenic shift, Antigenic drift, Epitope characterization, Heterosubtypic immunity, Homosubtypic immunity, Homologus immunity, Phage display, Bio-panning.

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INTRODUCTION

Influenza is one of the most frequent respiratory illness affecting humans, causing seasonal epidemic outbreaks every year. Despite the presence of a great number of influenza A subtypes, the isolates responsible of the global seasonal epidemics in the last decades belong basically to two different subtypes: H1N1 and H3N2 (Asner *et al.,* 2012; Baird *et al.,* 2012; Bartolini *et al.,* 2011; Centers for Disease Control and Prevention, 2012; WER, 2012). However, due to the "so-called" antigenic shift phenomenon, the emergence of new isolates causing pandemics represents an incumbent problem for the world population. In fact, in the past century three pandemic outbreaks have been responsible of many death and high economical costs worldwide. Nowadays, the only prophylactic or therapeutic measures available are the anti-influenza drugs such as the neuraminidase (NA) inhibitors and M2 blockers (adamantanes) and, the seasonal vaccination campaigns. Unfortunately, both antiviral drugs and "classical" vaccine preparations are burdened by several drawbacks. The main problems related to anti-viral drugs administration are represented by the emergence of resistant isolates due to aminoacid mutations on the viral NA and M2, and the need of their rapid administration to be effective (2012; Bauer *et al.,* 2012; Chintakrindi *et al.,* 2012; Govorkova and McCullers, 2012; Katsunuma *et al.,* 2012; Nguyen *et al.,* 2012; Uchimura *et al.,* 2012; Wang *et al.,* 2012; Zarogiannis *et al.,* 2012). Moreover, several associated side effects have been described, especially in high-risk categories (children and pregnant women) (Anar C *et al.*, 2010; Kitching *et al.,* 2009). Regarding the current vaccinal approaches, the main risks involved in their failure are basically the possible mismatch between the viral strains included in the seasonal vaccine preparations and

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the isolates circulating during epidemics. This, because the current influenza vaccine formulations are based on a process that involves the prediction of the most representative isolates circulating in the next influenza epidemic season. The "wrong" vaccine formulation predictions are basically due to the aptitude of influenza virus to undergo aminoacid mutation on its major surface glycoproteins (antigenic drift), resulting in its high variability able to elude the humoral protective response elicited by the vaccine. Obviously, the emergence of novel pandemic strains inevitably cannot be coped with the classical vaccinal approach (Blondel *et al.,* 2012; Nachtnebel *et al.,* 2012; Olivier, 2012). Given that, the need of novel anti-influenza prophylactic and therapeutic strategies are of primary importance. A key role for overcome these drawbacks can be played by the identification of human monoclonal antibodies (mAbs) able to target neutralizing epitope broadly shared among different influenza subtypes (Mancini *et al.,* 2004; Mancini *et al.,* 2011; Wang *et al.,* 2010). In this paper we will review the most promising broad range neutralizing human monoclonal antibodies directed against influenza HA to be used in clinical practice, as well as for the design of a novel epitope-based vaccine, underlining the importance of a fine epitope characterization (Figure 1).

Influenza hemagglutinin: a surface antigen characterized by its dual immunological propriety

The influenza HA is the most abundant influenza surface protein. To date, sixteen different types of HAs have been identified, however, only three of them (H1, H2 and H3) have been recognized to cause pandemics in humans. The 16 subtypes are further classified on the basis of phylogenetic analysis of their sequences in two groups. Group 1 (including H1, H2 and H5 subtypes), and group 2 (including H3 subtype) (Lambert and Fauci, 2010; Nabel and Fauci, 2010). HA is present on the viral envelope as an omo-trimeric structure. Each monomer is constituted by two HA subunits: HA1 and HA2 subunit. HA1 contains the HA globular head, in which are present the syalic acid binding sites responsible for the binding with the host cell receptors. The globular head on HA1 domain, is mostly exposed on the viral surface and is the most immuno-dominant region present within the molecule. Unfortunately, due to the massive immune response selective pressure elicited against the globular region, the B-cell epitopes present in this region undergo mutations responsible for the viral immune escape. HA2 is part of the so-called HA stem region, in which lies the fusion peptide allowing the post-entry membrane fusion between viral envelope and endosomal vescicle. The stem region of HA consisting in the whole HA2 region and part of HA1 region, is the most conserved region among the different HAs. It contains regions widely shared also among isolates belonging to phylogenetic group 1 and group 2 of HA (Skehel and Wiley, 2000). Nevertheless, these conserved regions are poorly immunogenic (Asner *et al.,* 2012) when presented to the host immune system in the HA naïve form (contained in the classical vaccine preparations and present on the viral surface as well).

Human neutralizing mAbs directed against HA

The viral neutralization of a mAb directed against HA can be mainly clustered into two different mechanisms. The first is the interference with the HA binding to the host cell receptor (this is the case of the Abs directed against the major antigenic HA sites), the second one, is the blocking of viral envelope fusion with the endosomal membrane (Skehel and Wiley, 2000). Obviously, the most abundant antibodies naturally produced against HA are mainly directed against the globular head and interfere with the antigen-receptor docking. The antibodies directed against the hypervariable antigenic sites, are usually endowed with strong neutralizing activity (homologus immunity) but their very limited neutralization range make them not useful from the clinical point of view. On the contrary, the Abs able to interfere with the fusion process, poorly induced by viral infection or vaccination as well, are endowed with broad range neutralizing activity against highly divergent influenza virus isolates, and represent the best candidates for a temptative use in therapy and prophylaxis.

Human monoclonal antibodies directed against HA-stem region

There are many mAbs directed against HA. However, only few of them can be considered pu-

tative candidates for human administration as already reported for the treatment or prophylaxis of other viral infections (Burioni *et al.,* 2008b). In fact, to be considered as good candidates for the human administration, they have to be endowed with some features. First of all, they have to be considered safe for humans. Secondly, they must be directed against widely shared HA epitopes, allowing their usage for the treatment of severe influenza illness caused by influenza isolates that can differ in their antigenic asset (heterosubtypic neutralizing activity). Thirdly, they should be endowed with strong neutralizing activity, allowing their *in vivo* use at low dosing.

Currently, only few research groups have described human mAbs endowed with such characteristics and endowed with heterosubtypic neutralizing activity as well (Table 1). Moreover, even if all the mAbs feature heterosubtypic neutralizing activity and recognize different conserved epitopes on the HA stem, the strategy adopted for their isolation is often completely different. In fact, the different strategies adopted span from the isolation from phage-display libraries (successful strategy widely used for the molecular cloning of mAbs directed against a plurality of microbial phatogens and allowing the selection of mAbs with different biological features) (Bugli *et al.*, 2011 Burioni *et al.,* 1998; Burioni *et al.,* 1994; Clementi *et al.,* 2012; Kashyap *et al.,* 2008; Mancini *et al.,* 2009; Sautto *et al.,* 2012a; Solforosi *et al.,* 2012; Sui *et al.,* 2009; Throsby *et al.,* 2008), or directly from human peripheral B-cells (Burioni *et al.,* 2010; Burioni *et al.,* 2009b; Corti *et al.,* 2011). This panel of human mAbs belongs to only three distinct VH-gene subfamilies: VH1-69, VH3-30 and VH3-23. More interestingly, all the mAbs directed against HAs belonging to phylogenetic group 1 are VH1-69 (gene usage often associated with autoimmune diseases) (Perotti *et al.,* 2008; Pos *et al.,* 2009; Sautto *et al.,* 2012b; Van Es *et al.*, 1992) except a single mAb (named PN-SIA49 and characterized by a very strong neutralization activity) directed against group 1 as well and belonging to VH3-23 subfamily (De Marco *et al.,* 2012). On the contrary, the only two human mAbs described to date in literature as able to neutralize viral isolates belonging to group 1 and 2 show a VH3-30 gene rearrangement. These mAbs, named FI6v3 and PN-SIA28 are both able to recognize pandemic strains and are both directed against HA-stem region, suggesting a post-entry inhibition of membrane fusion as neutralizing mechanism. Moreover, both mAb-epitopes have been well characterized even if using different approaches, suggesting the inclusion of a part of the fusion peptide as portion of mAbs epitopes (Clementi *et al.,* 2011; Corti *et al.,* 2011). In order to investigate the potency of these Abs several neutralization assays as well as *in vivo* studies have been performed (Clementi *et al.*, 2011; Corti *et al.,* 2011; De Marco *et al.,* 2012). Both PN-SIA28 and the human optimized FI6v3, showed a good neutralization potency even if, the techniques used for their potency calculation were not completely comparable. Together, all the results suggest that antibodies featuring similar characteristics can be stimulated by natural infection or by vaccination as well and, therefore, may represent a potential weapon, not only for new passive immunoprophylaxis strategies (Burioni *et al.,* 2009a; Mancini *et al.,* 2012), but also for new epitope-based vaccinal approaches setting-up (Burton and Parren, 2000; Karlsson Hedestam *et al.,* 2008; Nabel and Fauci, 2010). These mAbs will be briefly described in the fol-

TABLE 1 - *Human mAbs directed against conserved HA regions and endowed with broad neutralizing activity.*

mAb	*Isolation technique*	*V-gene germline*	*Specificity*	*References*
A06	Phage-display library	VH1–69	HA subtypes belonging to group 1	Kashyap et al.
CR6261		VH1–69		Throsby et al.
F10		VH1–69		Sui et al.
PN-SIA49	Human peripheral B-cells	VH3–23	HA subtypes belonging to group 1 and group 2	Burioni et al.
PN-SIA28		VH3–30		Burioni et al.
FI6v3		VH3–30		Corti et al.

lowing paragraph underlining their most important features.

A06

A06 (Kashyap *et al.,* 2008) was cloned from a human bone marrow library belonging to a patient infected by an avian strain of influenza virus belonging to H5N1 subtype and then selected using phage display technique. As shown in the Table 1, this mAb belong to VH1-69 gene rearrangement. It is able to recognize and neutralize both H1N1 and H5N1 influenza A subtypes (*Minimum Inhibitory Concentration* 9-83 µg/mL for H1N1 and 2-11 µg/mL for H5N1). Its efficacy was also tested *in vivo* using mouse models and its epitope encompasses aminoacid residues on the α-helix A of the HA2 on the stem region of HA.

CR6261

CR6261 (Throsby *et al.,* 2008) was cloned using phage display libraries from human healthy vaccinated donors. It uses a gene rearrangement VH1-69 (Table 1). It neutralizes viral isolates belonging to influenza Group 1 (IC_{50} for isolate belonging to H5N1 subtype: 0.55-3.71 µg/mL and for H9N2 5.24-14.87 µg/mL). It was also tested *in vivo* showing therapeutic efficacy (five days post infection) after challenges with strains belonging to H5N1 and H1N1 subtypes. Its epitope was identified through co-crystal resolution approaches and it lies on the stem region of HA.

F10

F10 (Sui *et al.,* 2009) was selected with H5N1-HAs from a phage display library obtained from a pool of peripheral B-cells from individuals not previously infected by H5N1 isolates. F10 showed *in vitro* neutralizing activity against isolates belonging to influenza Group 1 (H1, H2, H5, H6, H11), moreover it protects *in vivo* against lethal challenges of H5N1 virus. It target the HA stem region and its epitope mapping was performed by crystallization studies with H5N1 bound HA.

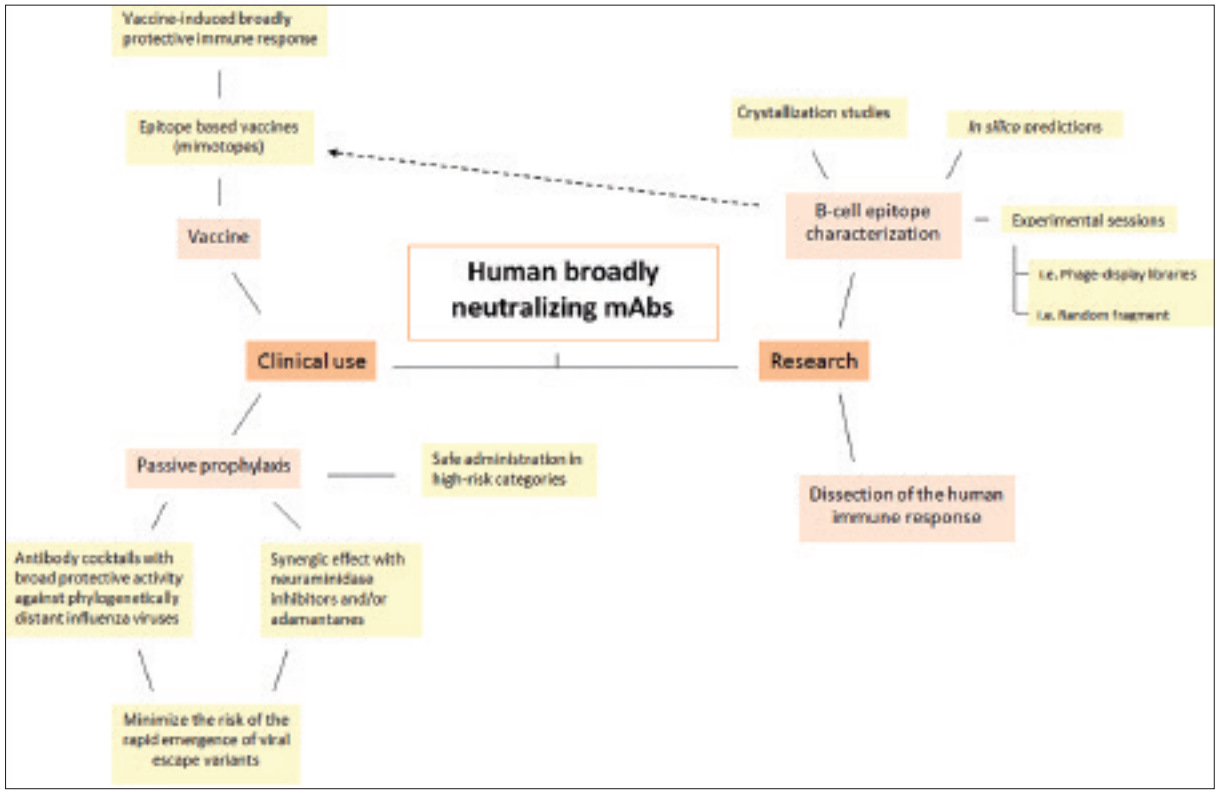

FIGURE 1 - *Principal uses of the most promising human mAbs directed against influenza isolates belonging to different subtypes: it is highlighted how a mAb can be a useful tool for both clinical use and research purposes.*

PN-SIA49
PN-SIA49 (De Marco *et al.*, 2012) was isolated from an healthy donor with negative clinical history for influenza in the last decade. It belongs to VH3-23 subfamily (Table 1) and recognizes a conserved epitope in the stem region of HA. PN-SIA49 features a very high *in vitro* neutralization potency against all the Group 1 tested strains except H9N2 avian tested strain (IC_{50} 0.2-1.9 µg/mL against H1, H2, H5). More importantly, it showed protective activity in mouse models when administrated twenty four hours after the lethal challenges with H1N1 and H5N1 isolates.

PN-SIA28
PN-SIA28 (Clementi *et al.*, 2011) was isolated from an healthy donor with negative clinical history for influenza in the last decade. It belongs to VH3-30 subfamily and recognizes a widely shared epitope among influenza HAs belonging to Group 1 and Group 2 isolates. PN-SIA28 epitope lies on the stem region of HA and encompasses HA1 and HA2 as well. Its biological activity was tested *in vitro* showing very low IC_{50} against the highly divergent tested isolates, comprising H1N1 2009 pandemic and avian H5N1 isolates (IC_{50} against Group 1 and 2 tested isolates 0.4-2.8 µg/mL).

FI6v3
FI6v3 mAb (Corti *et al.,* 2011) was isolated from peripheral B-cells from vaccinated patients. It belongs to VH3-30 subfamily and recognizes a broadly shared epitope located on the stem region of influenza virus Group 1 and 2. The FI6v3 epitope was identified by crystallization with bound HA. After its optimization, obtained through two mutations eliminating an isomerization site and a protease binding site, it was tested *in vitro* showing IC_{50} comprises between 1 and 12 µg/mL against the tested strains belonging to influenza phylogenetic Group 1 and 2. Its efficacy was assayed also *in vivo* against ferrets and mice.

CONCLUDING REMARKS

The availability of new human neutralizing monoclonal antibodies directed against widely shared epitopes on influenza hemmagglutinin represent a major advance to fight the severe influenza illness through their synergic usage with the antiviral drugs available to date (Figure 1). Nowadays, the panel of mAb showing similar features is restricted to few molecules (Clementi *et al.,* 2011; Corti *et al.,* 2011; De Marco *et al.,* 2012; Kashyap *et al.,* 2008; Sui *et al.,* 2009; Throsby *et al.,* 2008). These molecules are opening new perspectives in the field of therapy and prophylaxis of influenza infection and allowing the understanding of the virus-host interplay at a very deep level (Figure 1).
Moreover, these mAbs can also allow the design of novel vaccination strategies that can benefit of novel techniques such as the anti-idiotype generation approaches (Burioni *et al.*, 2008a; Karpatkin *et al.,* 1992; Zhou *et al.,* 1994; Zhou *et al.,* 1990) and the *in silico* design of new molecules mimicking the protective antigen region bound by the mAbs (Nabel and Fauci, 2010). Notwithstanding, the availability of mAbs such as those briefly described in this minireview could represent the starting point for the generation of new classes of antiviral drugs and new vaccines able to elicit a broad protective humoral immune response against a plurality of influenza isolates, even phylogenetically highly divergent each other.

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