Neutralization activity and kinetics of two broad-range human monoclonal IgG1 derived from recombinant Fab fragments and directed against Hepatitis C Virus E2 glycoprotein

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Hepatitis C virus (HCV) infects 170 million people worldwide and causes chronic liver inflammation and fibrogenesis leading to cirrhosis, end-stage liver failure and hepatocellular carcinoma (Alter, 1997; Seeff et al., 1992; Tong et al., 1995). HCV infection is also associated with several extra-hepatic complications, in particular to cryoglobulinemia (Perotti et al., 2008a; Sautto et al., 2012b). The development of an effective anti-HCV prophylactic or therapeutic strategy has been hindered by the ability of this virus to continuously mutate (Deutsch and Hadziyannis, 2008). The current standard treatment of HCV is based on a combination therapy (ribavirin and interferon) fraught with side-effects and associated with a low sustained virological response (50-55%), especially when used against the most common and “aggressive” HCV genotype (genotype 1) (Mira et al., 2009). Some hope has been recently stimulated by the introduction of anti-HCV protease inhibitors (boceprevir, telaprevir), but several studies have already reported a low mutational barrier against these drugs, especially when used in monotherapy, with the rapid emergence of resistant quasispecies (Forestier et al., 2007; Zhou et al., 2008). Overall, notwithstanding recent advances, there is still a great need for novel alternative anti-HCV strategies.

Increasing evidence supports the beneficial role of neutralizing antibodies in different viral infections (Burioni et al., 2010; Burioni et al., 2008; Clementi et al., 2011), including HCV (Chandra et al., 2010; Pestka et al., 2007; Yu et al., 2004). On these basis, the availability of anti-HCV monoclonal antibodies capable of targeting structurally and functionally conserved regions on the highly variable surface viral proteins is of extreme importance (Burioni et al., 2008; Johansson et al., 2007). In our laboratory, we previously isolated and characterized two broadly cross-neutralizing anti-HCV E2 human monoclonal Fab fragments named Fab-e20 and Fab-e137 (Mancini et al., 2009; Perotti et al., 2008b). These recombinant Fab fragments were obtained against HCV-E2 (Burioni et al., 1998b) using phage display-based

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strategies, a technique for cloning and optimization of monoclonal antibodies directed against human pathogens (Clementi et al., 2012; Mancini et al., 2004; Solforosi et al., 2012). However, the Fab fragment is characterized by a shorter half-life and is not endowed with many of the biological activities of the whole IgG1, that are often needed for a full exploitation of the clinical potential of the molecule (Jiang et al., 2011). In addition, the non-Fc-related biological activity of a Fab molecule often increases substantially when using it as a whole IgG (Clementi et al., 2011; De Marco et al., 2012). For this reason, the genes coding for the Fab molecules were used to produce the corresponding IgG1 molecules. In this paper, we describe the biological activity, the binding characteristics and the neutralization kinetics of these two novel anti-HCV human monoclonal IgG1.

The whole IgG1 antibodies IgG1-e20 and IgG1-e137 were generated from the corresponding Fab-e20 and Fab-e137 using the BD BaculoGold System (BD Biosciences Pharmingen), and purified as described (Sautto et al., 2012a) to a purity ≥95%. Reference Fabs were produced and purified as described (Burioni et al., 1998a). When tested in ELISA (Lesniewski et al., 1995), the two IgG1 molecules strongly reacted against HCV/E2 showing a 10-fold-higher affinity (ranging from 1 to 5 nM) than the corresponding Fab fragments against recombinant HCV/E2 protein derived from genotype 1a (strain H77) (data not shown). For a better definition of the cross-reactivity featured by the two antibodies, IgG1-e20 and IgG1-e137 were tested in immunofluorescence assay against human epithelial kidney (HEK) 293T cells expressing HCV E1-E2 from all six HCV genotypes (1a, 1b, 2a, 2b, 3, 4, 5, 6). Both IgG1 antibodies showed the capacity of binding all HCV genotypes tested, evidencing the highly conserved nature of their epitopes (data not shown). Remarkably, while Fab-e137 did not react against cells expressing genotype 5 glycoprotein, the corresponding IgG1-e137 acquired the ability to recognize also this genotype. This discrepancy can be due to the above mentioned fact that it is well known that human monoclonal Fabs directed against variable viral targets, once expressed in their "original" molecular structure, often acquire a wider reactivity (Clementi et al., 2011).

Fab-e20 and Fab-e137 epitopes were described as including conserved residues that are crucial for CD81 binding (Mancini et al., 2009; Perotti et al., 2008b), and it was important to demonstrate that this desirable feature was retained by the corresponding IgG1 molecules. As expected, IgG1-e20 and IgG1-e137 maintained the ability of in-

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IgG e20 IC50 µg/mL</th>
<th>Fab e20 IC50 µg/mL</th>
<th>IgG e137 IC50 µg/mL</th>
<th>Fab e137 IC50 µg/mL</th>
</tr>
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<tbody>
<tr>
<td>HCVpp 1a (H77)</td>
<td>0.02</td>
<td>7.5</td>
<td>0.03</td>
<td>5</td>
</tr>
<tr>
<td>HCVpp 1b (5.23)</td>
<td>0.3</td>
<td>&gt;15</td>
<td>0.9</td>
<td>20</td>
</tr>
<tr>
<td>HCVpp 2a (1.2)</td>
<td>1.2</td>
<td>7.5</td>
<td>1.6</td>
<td>N.D.</td>
</tr>
<tr>
<td>HCVpp 2b (1.7)</td>
<td>0.3</td>
<td>&gt;30</td>
<td>0.9</td>
<td>N.D.</td>
</tr>
<tr>
<td>HCVpp 3a (4.28)</td>
<td>1</td>
<td>N.D.</td>
<td>1</td>
<td>N.D.</td>
</tr>
<tr>
<td>HCVpp 4 (21.6)</td>
<td>1</td>
<td>1.6</td>
<td>1.4</td>
<td>5</td>
</tr>
<tr>
<td>HCVpp 5 (16.11)</td>
<td>0.7</td>
<td>N.D.</td>
<td>1</td>
<td>N.D.</td>
</tr>
<tr>
<td>HCVcc 2a (JFH1)</td>
<td>&lt;0.3</td>
<td>2.5</td>
<td>&lt;0.3</td>
<td>2.5</td>
</tr>
</tbody>
</table>

N.D. not determined.
interfering with the HCV/E2-CD81 binding. In particular at a concentration of 10 µg/mL the inhibition of binding was about 80% for both IgGs, higher than that observed with the corresponding Fabs (50%).

Obviously, the most important activity for an antiviral antibody is the capacity of blocking viral infectivity, and for this purpose IgG1-e20 and IgG1-e137 were comparatively tested against the corresponding Fabs against HCV pseudoparticles (HCVpp) derived from murine leukemia virus displaying unmodified and functional full-length E1-E2 proteins of genotypes 1 to 5 (Bartosch et al., 2003). Both IgG1-e20 and IgG1-e137 showed a strong neutralization activity against all tested genotypes (Table 1), with a remarkable increase of potency when compared with the corresponding Fabs. In particular, the neutralizing activity of both IgGs on HCVpp/1a was extremely high (Table 1), and IgG1-e20 and IgG1-e137 resulted to be the most powerful neutralizing anti-HCV monoclonal antibodies described to date.

Furthermore, the neutralizing activity of IgG1-e20 and IgG1-e137 was also evaluated using the cell culture infectious HCV (HCVcc) system based on HCV genotype 2a strain JFH-1 (Wakita et al., 2005). As expected from the HCVpp data, both antibodies showed a strong neutralizing activity also in this replicative HCV model (Table 1).

Antibody neutralization may involve different stages of viral infection, including attachment, internalization, or fusion. To identify the stage of viral cycle at which IgG1-e20 and IgG1-e137 interfere with the viral cycle, we used a pre- and post-attachment inhibition assay originally developed by Haberstroh et al. (Haberstroh et al., 2008). In this assay, the antibodies are incubated with HCVpp of genotype 1a before or after the binding to HuH-7.5 cells at 4°C, and the level of infection is then measured. IgG1-e20 and IgG1-e137 showed a pattern of inhibition similar to the one observed for antibodies directed against the viral cellular receptors CD81, confirming that the targeted entry step is closely linked to HCV-CD81 interaction (Figure 1A). To further investigate this aspect, we comparatively evaluated the inhibitory capacity of IgG1-e20, IgG1-e137, anti-CD81 antibody and heparin in a kinetic study in which, following protocol II, the inhibitory agents were added at various time points post-binding. This assay showed that IgG1-e20 and IgG1-e137 lost potency in blocking entry when added at 30 minutes post-attachment, suggesting that they interfere with the early post-binding steps of viral entry (Figure 1B).
In conclusion, we described two broadly cross-reactive and cross-neutralizing anti-HCV/E2 antibodies, IgG1-e20 and IgG1-e137. As the corresponding Fabs, IgG1-e20 and IgG1-e137 recognize a discontinuous epitope requiring residues within the CD81 binding site on E2 glycoprotein. Moreover, IgG1-e20 and IgG1-e137 showed similar kinetic of inhibition of HCV entry observed for anti-CD81 antibodies, indicating that they interfere at an early post-binding step. Moreover, an important issue in the role played by antibodies in the interplay between the host and HCV is neutralization interference by non-neutralizing antibodies (Burioni et al., 2001; Tarr et al., 2012; Zhang et al., 2009). Importantly, the two IgG1 described here have been described as not being affected by interfering antibodies in their neutralizing activity (Sautto et al., 2012a).

Our results demonstrate that IgG1-e20 and IgG1-e137 retain all desired features and have improved biological activity when compared to the corresponding Fabs. These data are encouraging in the quest for a potent cocktail of broad neutralizing monoclonal antibodies that could represent a novel anti-HCV therapy.

REFERENCES


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