論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	Pan Yang
論文題名 Title	Human monoclonal antibodies derived from a patient infected with 2009 pandemic influenza A virus broadly cross-neutralize group 1 influenza viruses （2009年パンデミックインフルエンザAウイルス感染患者由来ヒト型モノクローナル抗体は、グループ1に属するインフルエンザウイルスを広域に中和する）

論文内容の要旨

〔目　的(Purpose)〕

Influenza virus continuously threat to human public health because of their ability to evolve rapidly through genetic drift and reassortment. Vaccination is currently considered the best option to control influenza. However, this approach has several limitations, mainly because of viral antigenic changes, a phenomenon known as antigenic drift. A major limitation of current vaccine approach against a new pandemic is the time required to produce an appropriate quantity of antigenically matched vaccine. In addition, the emergence of resistance to antiviral drugs in recent years further limits the options available for the control of influenza. Therefore, we are now examining the prospect of new prophylactic and therapeutic approaches, including antibody therapy.

〔方法ならびに成績(Methods/Results)〕

PBMCs obtained from an H1N1pdm-infected patient at 3, 9, 16 and 23 days after the onset of influenza symptoms, were fused with SPYMEG cells to generate hybridomas. Ultimately, three hybridoma clones were selected: 1H11 and 5G2 were derived from PBMCs collected 9 days after onset of fever and 2H5 was derived from PBMCs collected 23 days after onset of fever. The cells secreted HuMAbs with a broad reactivity to group 1 influenza A viruses, including H1N1pdm, seasonal H1N1, H5N1 and H9N2. On the other hand, the HuMAbs did not react with the group 1 virus H2N2, group 2 influenza A viruses (H3N2 and H7N7) or influenza B virus. All the HuMAbs had little hemagglutination inhibition activity but had strong membrane-fusion inhibition activity against influenza viruses. A protease digestion assay showed the HuMAbs targeted commonly a short α-helix region in the stalk of the hemagglutinin. To investigate further the epitopes recognized by the three HuMAbs, we focused on the amino acid residues at positions 45 and 47 because the three HuMAbs distinctly neutralized the H1N1pdm 2009 strains, the 2011 strains and the H2N2 strain and these three virus groups differed in their α-helix residues at these two positions. We constructed four representative mutants of H1N1pdm 2009 HA, with Ile45Phe, Glu47Lys, Glu47Gly or Ile45Phe/Glu47Gly substitutions, and evaluated their reactivity with the HuMAbs. The three HuMAbs lost reactivity only when Ile45Phe/Glu47Gly double substitutions were introduced into the HA. This suggests that amino acids at positions 45 and 47 are critical for reactivity with the three HuMAbs. The sequences of the α-helix region of HA2 (at positions 40–58) recognized by the HuMAbs were retrieved from the NCBI IVR database and the sequences of several subtypes of influenza A viruses were compared. The result showed that, in general, only H2N2 viruses have simultaneous substitutions at positions 45 and 47. These two amino acid residues are highly conserved in the HAs of H1N1, H5N1 and H9N2 viruses.

〔総　括(Conclusion)〕

Thus, the HuMAbs reported here may be potential candidates for the future development of effective anti-influenza prophylactic and therapeutic antibodies.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

近年、インフルエンザに対する次世代の予防薬または治療薬の候補として、ヒト型抗体が研究されている。一方で、インフルエンザウイルス感染またはワクチン接種によって誘起される中和抗体の多くは、ヘマグルチニン（HA）頭部領域の抗原的な可変領域を認識するため、特定のウイルス株または亜型にだけ反応する。申請者らは、SPYMEGと呼ばれる新しいフュージョンパートナー細胞とインフルエンザ感染患者由来末梢血単核球を用いて、インフルエンザAウイルスに広域に反応するヒト型中和抗体（1H11, 2H5, 5G2）を産生するバイブリドーマ細胞を樹立した。1H11, 2H5および5G2は、HAを認識するにも関わらず、group 1に属するH1, H5およびH9亜型インフルエンザウイルスに広範な中和活性を示した。また、これらの抗体は、HAにおける抗原的な安定なstem領域に位置する新規エピトープを認識することを明らかにした。当該抗体および同定された新規エピトープの情報は、今後のインフルエンザ予防または治療薬の開発に大変有用である。これらの解析は、博士（医学）の学位授与に値すると考えられる。