論 文 内 容 の 要 旨

Synopsis of Thesis

氏 名 Name	Naphatsawan Boonsathorn
論文題名 Title	A human monoclonal antibody derived from a vaccinated volunteer recognizes heterosubtypically a novel epitope on the hemagglutinin globular head of H1 and H9 influenza A viruses (ワクチン接種健常者由来ヒト型抗体はH1およびH9亜型に属するインフルエンザAウイルスのHA頭部領域における新規エピトープを亜型間交差性に認識する)

論文内容の要旨

〔目 的(Purpose)〕

Vaccination is considered a promising control measure against pandemic and epidemic influenza but has several limitations, mainly because of viral antigenic drift. Furthermore, the emergence of resistance to antiviral drugs in recent years limits the options available for the control of influenza. Under such circumstances, the interest in human monoclonal antibodies (HuMAbs) as alternative prophylactic and therapeutic agents has been strengthened by the recent identification of heretrosubtypic neutralizing HuMAbs to influenza viruses. Most of these HuMAbs have been derived from phage display libraries and found to target the HA stem region. However, HuMAbs generated by this method are the products of random recombination between the immunoglobulin variable region heavy chain and light chain genes. In contrast, we have reported a cell-to-cell fusion method using a new fusion partner cell line, SPYMEG, and donor-derived peripheral blood mononuclear cells (PBMCs). In this study, we report the characterization of an anti-influenza HuMAb generated from the PBMCs of a volunteer vaccinated with seasonal influenza vaccine.

〔方法ならびに成績(Methods/Results)〕

Method: PBMCs were obtained from a healthy volunteer 7 and 14 days after receiving the trivalent seasonal influenza vaccine. The PBMCs were fused with SPYMEG cells to generate hybridomas. Ultimately, a hybridoma clone designated 5D7, which was derived from the PBMCs collected at 7 days after vaccination, was selected.

Result: The 5D7 secreted HuMAb that heterosubtypically cross-reacted with group 1 influenza A viruses, including seasonal H1N1, H1N1pdm and H9N2. In contrast, the HuMAb did not react with other group 1 viruses (such as H2N2 and H5N1), group 2 viruses or influenza B viruses. In accordance with the reactivity, the HuMAb showed cross-neutralizing activity against seasonal H1N1, H1N1pdm and H9N2 influenza virus strains. An HI assay showed that the HuMAb had HI activity against both seasonal H1N1 and H1N1pdm. Western blotting analysis also showed that the HuMAb recognized A/Suita/1/2009 HA under non-reducing conditions but had no such reactivity under reducing conditions. Selection of escape mutant showed that the HuMAb recognizes a novel epitope region (residues 112–118) in the HA head region, which is distinct from epitopes recognized by the heterosubtypic neutralizing HuMAbs against influenza viruses reported previously. A database search also revealed that the epitope sequences are highly conserved among H1 and H9 viruses.

〔総 括(Conclusion)〕

Several heterosubtypic neutralizing HuMAbs with broad spectrum activity against influenza A viruses have been described recently. Most of the HuMAbs were isolated from phage display libraries and recognized conserved epitopes located in the HA stem region. In contrast, information about conserved epitopes located in the HA globular head region is rather limited. Thus, it is not known whether equivalent HuMAbs that target the globular head region are produced in the course of the immune response to influenza virus. The main finding of this study is that heterosubtypic neutralizing IgG antibodies that target HA globular head can be elicited in humans following exposure to seasonal influenza vaccine. In addition, Most heterosubtypic cross-neutralizing HuMAb have been reported to recognize epitopes located mainly in the most conserved HA stem region. To our knowledge, 5D7 is the first heterosubtypic neutralizing HuMAb that targets a conserved epitope distinct from the RBS in the HA globular head region. Thus, the HuMAb reported here may be a potential candidate for the future development of effective anti-influenza prophylactic and therapeutic antibodies. In addition, our findings of a novel epitope that is highly conserved in the HA globular head of H1 and H9 influenza viruses should provide useful information for designing and developing a universal vaccine against influenza viruses.

論文審査の結果の要旨及び担当者

（申請者氏名）ナパサワン ブーンサトン（Naphatsawan Boonsathorn）

論文審査担当者	（職）	氏名
主査	大阪大学招へい教授	生田和良
副査	大阪大学教授	塩田達雄
副査	大阪大学教授	松浦善治

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ワクチン接種健常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（5D7）を産生するバイブリドーマ細胞を樹立した。5D7は、HA頭部領域を認識するにも関わらず、group 1に属するH1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、5D7はレセプター結合部位からやや離れた新しい構造エピトープを認識することを明らかにした。5D7および当該抗体が認識する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。