pointed out in the membrane-blotting method does not hamper the immunogel-biopanning method. In the case of one cfu of the h18-108 scFv-phage in the phage solution (Fig. 4c), the enrichment was not shown even in the fourth round of biopanning. This might be attributed to non-specific loss due to the extreme case of 1 cfu of a specific clone.

DISCUSSION

Filamentous phages attach non-specifically to a Western blotting membrane, making it difficult to enrich antigen-specific phage antibodies from phage libraries.[3,4] Liu *et al.* reported that methanol-treatment of the polyvinylidene fluoride (PVDF) membrane could overcome this difficulty.[3] However, there have been few reports of success in the discovery of new protein molecules using this method.

In our hands using IL-18, Liu's protocol gave 1 to 2×10^6 phage-recovery (background) after the phage-amplification eluted from 25 mm^2 PVDF membrane coated with or without IL-18, even if the library contained no IL-18-specific phage clones. Therefore, we did not perform the experiments to compare quantitatively to Liu's method using purified IL-18 or its mixture with unrelated proteins. The purpose of this study was, rather, to develop the panning procedure with far less non-specific phage-binding and determine the minimum amount of target molecule in a sample that may result from a total loss occurring after experimental manipulations, such as electrophoresis, gel slicing, elution efficiency, protein coating, or sensitivity of ELISA.

To simulate the practical experimental conditions, the IL-18 was mixed with the cell lysates and analyzed for feasibility. The cell lysates contained 6.4 μg/8 μl/lane which was the highest amount of sample applied in our experiments. We showed first that immunogel-biopanning successfully worked when the cell lysates contained 50 ng of the target molecule. The amount of 50 ng can be reduced if an experimental apparatus or a manipulation method is devised. Secondly, this method works when the phage solution contains 10 cfu of target-specific phage clones in a total of 10^{11} cfu of an unrelated phage population. This frequency of a target-specific clone is quite usual in phage libraries. As the unrelated phages were a mixture of several clones of non-IL-18 binding phage, therefore, this result was attributed to the specific clone selection *via* anti-IL-18 scFv displaying on a phage surface but not the non-specific binding *via* non-scFv phage surface molecules. This result indicated that 10 out of 10^{11} phages were able to detect 5 ng of the target protein per well by this procedure (Figs. 3, 4). It is noteworthy that a target protein that is invisible by even silver staining can be accurately detected by antibody-phage propagation. It is also of interest that the amplification of the detection sensitivity is accomplished by phage propagation, while in other methods it is usually attained by enzymatic chemical reactions or electrical manipulations.

Another salient feature of our experiment is that this method was attained using IL-18 conformation-specific scFv phage clones. Biopanning using Western blotting is not suitable for isolation of conformation-specific phage clones. It is well known that the conformation of protein is critically related to severe pathogenesis such as in amyloid β or prion proteins.[10,11] In these cases, the antibodies specific to various conformers are promising tools for analyzing the pathogenesis of protein conformation diseases and development of immunotherapeutic reagents. The immunogel-biopanning may be useful for this purpose. We are isolating scFv-phage clones specific to a distinctive conformer, Barghorn's globulomer of amyloid β_{1-42},[12] from the mixture of various conformers, employing this immunogel-biopanning method [in preparation].

Thus, immunogel-biopanning is useful for the isolation of a monoclonal antibody specific to a rare target molecule using crude mixtures of tissue or cell lysates. This method may also be promising for quantitative purposes and have much higher sensitivity if it is combined with the recently reported immuno-polymerase chain reaction method.[13]

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班会議プログラム

厚生労働科学研究費補助金(こころの健康科学研究事業)班会議

[プリオン病における免疫反応の解明とそれに基づく診断・治療法の開発]

平成19年度班会議プログラム

開催日	平成２０年３月３日（月）
時間	15：00～
場所	長崎大学　医学部　ポンペ会館　第１会議室

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   石橋大輔先生（長崎大学大学院医歯薬学総合研究科感染分子解析学）

2. 「培養細胞系でのプリオン感染 One-cell-detection の試み」
   山口尚宏先生（長崎大学大学院医歯薬学総合研究科感染分子解析学）

3. 「コンフォメーションを保持した全長プリオン蛋白を識別するヒト抗体」
   杉村和久先生（鹿児島大学生体工学科分子生物工学講座）

4. 「抗プリオン蛋白抗体 scFv による抗プリオン活性」
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