1P137**Computational prediction of T cell epitope peptides for designing hepatitis C virus vaccine.**○Keiko Uda¹, Toshie Mashiba², Tomoya Miyakawa³, Tsutomu Osada³¹Dept. of Immunology, Kochi Medical School, ²Dept. of Gastroenterology and Metabolism, Graduate School of Medicine, Ehime Univ., ³Dept. of Bioinformatics Business Promotion, NEC corporation

Cytotoxic T lymphocytes (CTLs) play a pivotal role in eradicating hepatitis C virus (HCV) infected cells. CTLs recognize HCV derived peptides that are presented by HLA (human leukocyte antigen) class I molecules. Since HLA molecules display extensive polymorphism presented peptide varies from patient to patient. In order to develop an effective vaccine it is necessary to identify HCV peptides that bind to host's HLA molecules. We have previously developed a computational method to analyze the sequence rules that govern the allele specific peptide binding to HLA molecules and a program to predict binder peptides. By using the program we identified a number of high binder peptides for HLA-A*2402 molecule a most frequently found allele among oriental populations. HLA binding was examined experimentally to confirm actual binding. Peptides were then tested whether they can stimulate and expand specific CTLs from PBMC (peripheral blood mononuclear cells). Except for a few peptides cytotoxic T cell repertoire could be expanded by stimulation with peptides in vitro. The biggest challenge to develop peptide based vaccine for HCV lies in its frequent mutation that leads to immune evasion. Since evolutionary examination of the viruses circulating Japanese archipelago revealed no particular bias for immune evasion in HLA-A*2402 binding sequences a cocktail vaccine was designed to root out variant viruses before they acquire further mutations. An ease in identifying HLA binding peptides should help design targeting peptides for immunotherapy.

1P139**BmNPV expression system; Application to human natural killer cell receptors**○Mizuho Kajikawa¹, Kaori Sasaki¹, Kimiko Kuroki¹, Tomoko Motohashi², Tsukasa Shimojima², Enoch Y. Park³, Daisuke Kohda¹, Katsumi Maenaka¹¹Div. Struct. Biol., Med. Inst. Bioreg., Kyushu Univ., ²Nat. Inst. Genetics, ³Dept. Appl. Biol. Chem., Shizuoka Univ.

Silkworm expression system is attractive for large-scale protein production because of (1) strong polyhedrin promoter, (2) sugar modification, (3) no biohazard. The commercially available baculovirus expression system is based on AcNPV virus, which is not infectious for silkworms. Its bacmid DNA can be replicated in both *E. coli* and insect cells, and thus time-consuming and technically difficult plaque assay is not required. However, there was no bacmid system for BmNPV, which can directly infect silkworm, until recently we established the BmNPV bacmid system. Here we report the silkworm large-scale protein production of human natural killer cell receptors using the BmNPV bacmid system. The ectodomains of killer cell Ig-like receptor 2DL1 (KIR2DL1), KIR2DL4 and CD160 with histidine tag at the C-terminal site were cloned in pFastBac transfer vector (Invitrogen) and they were recombined into the BmNPV bacmid DNA in *E. coli*. The recombinant virus genome DNAs were directly injected into silkworms. 5 days after the injection, the expression of the sugar-attached ectodomains in body fluid was confirmed by western blotting using anti-penta His antibody. Furthermore, we successfully purified the KIR2DL1 ectodomain by Ni-NTA affinity chromatography and the yield is roughly 0.2 mg/l larvae. In conclusion, the BmNPV bacmid system is easy-to-use, quick and suitable for large-scale production of sugar-modified eukaryotic proteins. Therefore, it is powerful method for future proteomic studies including structural studies and vaccine productions.

1P138**A method for producing of rabbit monoclonal antibody from spleen and bone marrow B cells using yeast display system**

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Recently, the rapid development of protein specific antibodies is required to identify the function and localization of proteins. Monoclonal antibodies are established from mice or rats spleen cells by hybridoma technology. However, sometimes we can not obtain good monoclonal antibodies from these animals. On the other hands, rabbit is well known as a good antibodies producer. Usually, rabbit produces high affinity polyclonal antibodies. We tried to establish the method for producing of rabbit monoclonal antibody using yeast display system. The messenger RNAs were corrected from spleen and bone marrow B cells of antigen immunized rabbits, respectively. The heavy and light chain variable fragments of antibodies were amplified separately. The single chain variable fragments of antibodies (scFv) DNA were assembled from these fragments and the scFv were expressed on the yeast surface. The yeast expressed antigen specific scFv were collected by MACS technology and isolated by FACS sorting. After plating the isolated yeast, single colonies were picked up, grown and scFv DNA were extracted. The scFv DNA were inserted into the expression vector and expressed by *Saccharomyces cerevisiae*. The scFv were analyzed by ELISA. From the results, we confirmed the establishment of the system to obtain the good rabbit monoclonal antibodies. We also report the comparison between the scFv from spleen and bone marrow B cells.

1P140**A novel expression system for high-level recombinant protein production in *Escherichia coli***○Eri Hojo¹, Masakatsu Kamiya^{1,2}, Tomoyasu Aizawa^{1,2}, Mitsuhiro Miyazawa³, Yusuke Kato³, Makoto Demura^{1,2}, Keiichi Kawano¹¹Grad. Sch. of Sci., Hokkaido Univ., ²Grad. Sch. of Life Sci., Hokkaido Univ., ³Dept. of Dev. Biol., Natl. Inst. of Agrobiol. Sci.

One of the key steps of structural genomics and proteomics is to produce target proteins. Many protein expression systems, including *Escherichia coli*, Yeast, Baculovirus-infected insect cell, mammalian cell, and cell-free protein synthesis, have been established to produce recombinant proteins. Moreover, a wide variety of strategies have been developed to improve the yield of recombinant proteins. One of such strategies is fusion protein. Generally, fusion protein system has been used to improve protein folding and solubility, resulting in obtaining biologically active products. However, these approaches are not always successful. In particular, it is very difficult to produce target proteins that are lethal to host cell and/or easy to be degraded in soluble forms. We present here a novel method to facilitate the expression level in *E. coli* of recombinant protein that is difficult to express in conventional production system. We demonstrated that coexpression of the aggregation-prone protein remarkably enhanced the expression level of target protein. It seems that over-expression of the partner protein protects target protein from proteolytic degradation by forming insoluble inclusion bodies and accounts for the higher observed yields. Importantly, it is possible to easily isolate target protein from partner protein by chromatography, and never requires chemical or enzymatic cleavage of fusion protein. This advantage makes our new method more effective and cost-efficient, expandable to large-scale production.