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# Abstracts

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## Hyaluronic Acid Target Receptors (CD44 and RHAMM) involved in treatment of type 1 diabetes

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We have shown earlier that the development of type 1 diabetes (T1D) in NOD mice is highly dependent on interaction between cell surface CD44 and its counter-molecule hyaluronic acid (HA) (Weiss et al., Proc. Natl. Acad. Sci. USA. 97, 285–290, 2000). However, the mechanism of action underlined this finding has not been elucidated. We show here that the balance between CD44-dependent low mobility of inflammatory cells and CD44-dependent high susceptibility of $\beta$ cells to apoptosis dictates the development of T1D in NOD mice. CD44-positive (wild type) inflammatory cells are less mobile than the corresponding CD44-deficient cells, because they firmly bind to the HA substrate. Yet, this anti-diabetic effect is counter-balanced by the enhanced susceptibility of CD44-positive insulin-secreting $\beta$ cells to the autoimmune attack, while CD44-deficient $\beta$ cells show relative resistance to this pathological activity. The reduced mobility of CD44-positive diabetic inflammatory cells has been verified by findings showing that they invade the pancreatic islets less intensively and cross HA-coated filters at lower rates than corresponding CD44-deficient cells. On the other hand, the relative resistance to cytokine-mediated apoptosis by CD44-deficient $\beta$ cells has been proven by reduction of inducible nitric oxide (NO) synthase (iNOS) and caspase-3 signals, as well as by decline in their NO release and increase in glucose-stimulation insulin secretion, when compared to corresponding wild type cells. Pancreatic islet cells removed from CD44-deficient pre-diabetic NOD mice showed also relative resistance to apoptosis, when compared with corresponding wild type mice, as indicated by reduction of iNOS and caspase-3 signals. Hence, the balance between the reduced mobility of CD44-positive inflammatory cells and the enhanced susceptibility of $\beta$ cells to programmed cell death dictates whether the mouse is diabetic or free of diabetes. In CD44 knock out female NOD mice the net balance favor resistance of $\beta$ cells to apoptosis over the enhanced mobility of the inflammatory cells, while the opposite is true for wild type mice. Cell surface RHAMM (CD168) allows inflammatory cell mobility when CD44 is genetically deleted, because it loosely binds to HA.

## Surface IgM stimulation induces MEK1/2-dependent MYC expression in chronic lymphocytic leukemia cells

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Although long considered as a disease of failed apoptosis, it is now clear that chronic lymphocytic leukemia (CLL) cells undergo extensive cell division *in vivo*, especially in progressive disease. Signalling via the B-cell receptor is thought to activate proliferation and survival pathways in CLL cells and also has been linked to poor outcome. Here we have analysed the expression of the proto-oncoprotein MYC, an essential positive regulator of the cell cycle, after stimulation of surface IgM (sIgM). MYC expression was rapidly increased after sIgM stimulation in a subset of CLL samples. The

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ability of sIgM stimulation to increase MYC expression was correlated with sIgM induced intracellular calcium fluxes. MYC induction was partially dependent on the MEK/ERK signalling pathway, and MYC and phosphorylated ERK1/2 were both expressed within proliferation centers *in vivo*. Although stimulation of sIgD also resulted in ERK1/2 phosphorylation, responses were relatively short lived compared with sIgM and were associated with significantly reduced MYC induction, suggesting that the kinetics of ERK1/2 activation is a critical determinant of MYC induction. Our results suggest that ERK1/2-dependent induction of MYC is likely to play an important role in antigen-induced CLL proliferation. (Blood. 2012; 119(1):170-179).

## High affinity IgG antibodies develop naturally in Ig-knockout rats carrying germline human IgH/Igk/Igl loci bearing the rat $C_H$ region

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In mice transgenic for human immunoglobulin (Ig) loci, suboptimal efficacy in delivery of fully human antibodies has been attributed to imperfect interaction between the constant regions of human membrane IgH chains and the mouse cellular signaling machinery. To obviate this problem, we describe a humanized rat strain (OmniRat$^{TM}$) carrying a chimeric human/rat IgH locus together with fully human light-chain loci. The endogenous rat Ig loci were silenced by designer zinc finger nucleases. Following immunization, OmniRats performed as efficiently as normal rats in yielding high affinity serum IgG. Monoclonal antibodies, comprising fully human variable regions with sub-nanomolar antigen affinity and carrying extensive somatic mutations, are readily obtainable – similarly to the yield of conventional antibodies from normal rats.

## Cross-neutralizing activity of human anti-HIV-1 monoclonal antibodies do not correlate with the percentage of mutations in their variable fragments

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Antibodies (Abs) specific to HIV-1 envelope regions, the CD4 binding domain (CD4bd), V3 and V2 loops, which contain binding sites for cellular receptors and co-receptors display neutralizing activities and may have protective functions. These Abs are present in most HIV-infected subjects and can also be induced by vaccines but exhibit rather low neutralizing activities, which could be related to their incomplete affinity maturation. The Abs adapt to antigens through a number of mutations in the variable fragment of the heavy and light chain genes. Although the frequency of mutations may have a wide variation in matured Abs, it is quite meaningful that broadly neutralizing monoclonal Abs (mAbs) such as VRC01, PG9/16 and the series of PGT mAbs contain a substantially increased percentage of mutations, in the range of 30% to 45%, in their variable fragments. There is not much data about mutations in Abs induced by vaccines and by natural infection which are cross-neutralizing. We have studied the percentage of mutations in 63 human mAbs, generated using cellular methods, which are specific to CD4bd, V3 and V2 domains and looked for relationships with the neutralizing activities tested against a standard panel of 41 pseudoviruses (psVs).

All mAbs neutralized, with various frequencies (2% to 39%), the same panel of 8–9 tier 1 sensitive psVs and a few more resistant tier 2 psVs out of 41 psVs tested. The percentage of mutations in the nucleotide sequence was relatively low, in the range of 6.7% to 9% for the heavy chains and 3% to 5.9% for the light chains. A significantly increased percentage of mutations compared to mAbs derived from healthy, non-HIV-1 infected subjects was observed in FR 1, 2, 3 and CDR H1 but not in CDR H2 domains of variable fragments of the mAbs. In contrast to expectations, no significant correlation was observed between the percentage of mutations and cross-neutralization of mAbs. The relatively small percentage of mutations and different mAbs' epitopes may partly explain the lack of correlation. The results indicate that human HIV-1 neutralizing Abs specific to CD4bd, V3 and V2 domains require relatively low rates of mutations, which can probably be achieved during vaccination and contribute to protection against HIV-1 infection.

## Genetic immunization, an alternative for generating therapeutic antibodies

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Over the last 20 years, antibody-based immunotherapy has shown very promising results in the battle against cancer and against many other diseases. Monoclonal antibodies reveal great benefits over small chemical entities (SCE) as they specifically recognize their targets whilst leaving the surrounding tissue intact. Furthermore, because of their high specificity, antibodies have more predictable toxicity than SCEs. Antibodies can also stimulate effector mechanisms within the body that represent natural endogenous defense mechanisms, e.g., ADCC, complement activation etc., as well as potentially modulating function of e.g., cell surface receptor proteins.

Generating promising therapeutic antibodies is a critical step as these must specifically recognize their targets in their native conformation. Aldevron Freiburg proposes its proprietary GENOVAC Antibody Technology as an important tool to generate highly specific custom-made high affinity antibodies against native proteins directly from cDNA (genetic immunization). The main targets of interest for immunotherapy by antibodies are cell surface receptor proteins and their ligands as these are more accessible by systemically applied therapeutic antibodies compared to intracellular proteins. Based on 14 years of experience, we have developed this technology to cater for all protein targets where other methods have failed (e.g. GPCRs, plus other difficult cell surface receptor proteins). The technology utilizes the natural cellular secretion pathways of the immunized animals (mice or rats), whereby cell surface and secreted proteins are correctly folded and post-translationally modified as they would be in the human body. Several of the antibodies thus far produced using this technology, have also been shown to modulate function of the target proteins. GENOVAC genetic immunization technology has been referenced in over 140 publications and patents and can be applied to a wide range of targets.

Aldevron Freiburg, a leader in the field of genetic immunization, shows examples of projects where its proprietary GENOVAC Antibody Technology has been successful in generating antibodies that could be further developed as therapeutics. Indeed, some are already in clinical development.

## Improved antibody therapies for the treatment of Clostridium difficile infection

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*Clostridium difficile* infections (CDI) remain a major health and economic burden in care facilities of the developed world in spite of the availability of effective antibiotic therapies. Humanised IgG1 antibodies were generated by animal immunisation followed by direct rescue of antibody variable regions from B-cells. Antibodies were tested in a wide range of *in vitro* and cell culture assays in order to stratify them on affinity, neutralisation activity, protection against TEER loss and biophysical characteristics. A mixture of purified antibodies was tested in a hamster infection protection model and compared directly against synthesised versions of the Medarex (licensed to Merck) Mabs previously called CDA1 and MDX1388 (MK-3415A). The UCB Mab mixture conferred 100% protection during the first 11days of the acute infection phase and 82% protection out to 28 days during the chronic/re-infection phase. The Medarex (Merck) Mabs conferred substantially lower levels of overall protection. These differences in efficacy are rationalised by supporting *in vitro* comparisons of toxin affinity, neutralisation activities, binding stoichiometries and relative activities against TcdA and TcdB.

Potent UCB Mab mixtures may have potential as a new treatment of patient symptoms which are currently inadequately served by current therapies. In particular UCB Mab mixtures may have the potential to reduce death rates and reduce the duration and severity of diarrhoea in addition to further reducing recurrence rates. Improvement in these aspects would have a significant positive influence on patients and healthcare providers.

## Development of catalytic human antibody light chains (Antigenases) showing suppression of rabies virus infection

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By immunizing ground-state peptides or proteins into mice, the authors have established some catalytic antibody light chains (murine antigenases) that could destroy, (i) HIV-1 gp41, (ii) chemokine receptor CCR5

peptide [1], (iii) *Helicobacter pylori* urease etc. [2], (iv) TNF alpha [3] and (v) influenza virus [4]. Based on their structural analysis, we proposed a concept [5], in which the antigenase encodes a catalytic triad-like structure composed of Ser, His and Asp in some characteristic germline genes such as cr1, cs1, bd2 etc. These amino acid sequences have a high homology to those of human germline genes of subgroup II in kappa light chain. It is our ultimate goal to develop new therapies for human patients by applying the above concept and utilizing the advantage of human catalytic antibody.

Based on the above concept, we amplified and cloned cDNAs encoding the human antibody light chains (kappa) belonging to subgroup II, which were prepared from leukocytes of a volunteer vaccinated with rabies virus. The obtained cDNAs were transformed into *E.coli* and each human light chain was expressed as the protein, followed by the purification. The highly purified (over 95%) human light chains were submitted to investigate the ability whether or not they can suppress the infection of rabies virus against NA cells *in vitro* assay or *in vivo* assay. Remarkably, #18 clone suppressed the infection of both CVS and HEP strains of rabies viruses *in vitro* assay. In addition, we performed *in vivo* assays using ddY mice. The administered mice with rabies virus treated with #18 antigenase enhanced the number of survival mice compared with non-treated mice. Interestingly, #10 antigenase showed little effect on the suppression of the infection *in vitro* assay, but it exhibited the suppressive effect on the infection *in vivo* assay. These data suggested that the human antigenases could be developed as a new drug for therapy of humans in future.

## References

[1] T. Uda et al., *Biotechnol Bioeng* **86** (2004), 217–225.
[2] E. Hifumi et al., *J Biol Chem* **283**(2) (2008), 899–907.
[3] E. Hifumi et al., *FEBS J* **277** (2010), 3823-3832.
[4] E. Hifumi, T.Uda et al., *J Am Chem Soc* **133**(38) (2011), 15015–24.
[5] T. Uda et al., *J Biosci Bioeng* **97** (2004), 143–152.

## An effective approach for the generation and characterization of neutralizing monoclonal antibodies to viral targets

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Monoclonal antibodies (mAbs) against viral antigens have become one of the most significant biologics for target validation and drug development for infectious diseases. The generation of functional mAbs specific for viral targets, however, is hampered by the difficulty of using a living infectious agent as immunogen and by the ineffective methods for identifying mAbs that recognize native viral epitopes. Here we have developed an effective approach to generate and screen high-quality mAbs against targeted antigens of human cytomegalovirus (CMV). In the present study, the CMV antigens of interest were molecularly cloned and expressed stably on the cell surface of a mouse pre-B cell line that was derived from a Balb/c strain, and the transfected cells were then used as antigens for immunization of Balb/c mice and for subsequent mAb screenings. Hybridoma clones were generated from the fusion of a murine myeloma cell line with splenocytes from the immunized mice. Specific hybridoma clones were differentially screened against the CMV antigen-expressing transfectants versus the parental non-transfected cells by flow cytometry in a high-throughput mode. By utilizing this technique, large panels of CMV-specific mAbs have been generated and identified. Our results indicate that the key benefits of this cell-based immunization strategy are the dramatically improved immune responses in animals to the CMV targets, considerable increase in the yield of mAbs that bind viral antigens in their native form, and complete circumvention of any purification process for viral proteins. More importantly, several anti-CMV mAbs generated by this approach have functional activity and 4 out of the 26 mAbs blocked at least 90% of virus binding to their receptors on human fibroblast and epithelial cells, as determined by an *in vitro* neutralization assay. The technical platform established from this work enables us to answer unmet needs for high-quality mAbs in discovery research and therapeutic development.

## Immune complex vaccines to elicit antibodies against HIV-1 neutralizing epitopes

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The development of HIV vaccines that elicit potent neutralizing antibodies against a broad array of

HIV-1 isolates faces formidable challenges. Although broadly reactive neutralizing epitopes on HIV-1 envelope antigens gp120 and gp41 have been mapped, these epitopes are poorly immunogenic. Hence, novel immunogen designs to augment the immunogenicity of these neutralizing epitopes are warranted. We have established a unique immunogen design strategy that exploits immune complexes of gp120 and selected anti-gp120 monoclonal antibodies (mAb). Immunization with gp120/mAb complexes, but not uncomplexed gp120, elicited antibody response against neutralizing V3 epitopes. This capacity was dictated by fine specificity and affinity of mAbs used to form the complexes, indicating the contribution of Fab-mediated activity. Further improvement of V3 immunogenicity was attained by forming immune complexes with gp120 mutants lacking site-specific N-linked glycans and by priming with DNA encoding the same gp120 mutants. Increased V3 immunogenicity on the mutated gp120/mAb complexes correlates with enhancement of *in vitro* antigenicity and proteolytic resistance of V3 epitopes when presented on the complexes. To target viruses bearing different V3 variants, immune complexes made of gp120 from different HIV-1 strains were tested and found to stimulate distinct and complementary neutralizing Ab responses. Hence, gp120/mAb complexes are superior immunogens for eliciting high titers of anti-gp120 Abs and directing the Ab response against neutralizing epitopes, and a cocktail of complexes has the potential to broaden neutralizing Ab responses against a larger array of HIV-1 isolates.

## Immunogenic mechanisms driving norovirus G11.4 antigenic variation

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Noroviruses are the principal cause of epidemic gastroenteritis worldwide with GII.4 strains accounting for 80% of infections. The major capsid protein of GII.4 strains is evolving rapidly resulting in new epidemic strains with altered antigenic potentials. To test if antigenic drift may contribute to GII.4 persistence, human memory B cells were immortalized and the resulting human monoclonal antibodies (mAbs) characterized for reactivity to a panel of time-ordered GII.4 virus-like particles (VLPs). Reflecting the complex exposure history of the volunteer, human anti-GII.4 mAbs grouped into three VLP reactivity patterns; ancestral (1987–1997), contemporary (2004–2009), and broad (1987–2009). NVB 114 reacted exclusively to the earliest GII.4 VLPs by EIA and blockade. NVB 97 specifically bound and blocked only contemporary GII.4 VLPs, while NBV 111 and 43.9 exclusively reacted with and blocked variants of the GII.4.2006 Minerva strain. Three mAbs had broad GII.4 reactivity. Two, NVB 37.10 and 61.3 also detected other genogroup II VLPs by EIA but did not block any VLP interactions with carbohydrate ligands. NVB 71.4 cross-neutralized the panel of time-ordered GII.4 VLPs, as measured by VLP-carbohydrate blockade assays. Using mutant VLPs designed to alter predicted antigenic epitopes, two evolving, GII.4-specific, blockade epitopes were mapped. Amino acids 294–298 and 368–372 were required for binding NVB 114, 111 and 43.9 mAbs. Amino acids 393–395 were essential for binding NVB 97, supporting earlier correlations between antibody blockade escape and carbohydrate binding variation. These data inform VLP vaccine design, provide a strategy for expanding the cross-blockade potential of chimeric VLP vaccines, and identify an antibody with broadly neutralizing therapeutic potential for the treatment of human disease. Moreover, these data support the hypothesis that GII.4 norovirus evolution results in antigenic drift of neutralizing epitopes and consequently, antibody-driven receptor switching; thus, protective herd immunity is a driving force in norovirus evolution.

## Human antibody light chains (antigenases) showing cytotoxicity against cancer cells

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The authors have reported in HAH2009 and HAH 2011 about the preparation and expression of human catalytic antibody light chains (antienases) possessing catalytic activity (amidase activity) which were mostly encoded in Subgroup II in kappa type. In HAH 2011 conference, we introduced an interesting results in which #7 antigenase showed cytotoxicity against A549 cells (lung cancer purchased from ATCC). In addition, it seemed that the antigenase did not kill the A549 cells but suppressed the proliferation of the cells.

We obtained the human antigenase gene (human kappa light chain presumably possessing some catalytic activities) by a semi-nested PCR using cDNA prepared from human leukocyte as a template. The obtained 18 kinds of the genes (wild type) and mutants (C220A) of a monomeric form of the antigenase were transformed in *E.coli* and the expressed proteins were recovered and highly purified by employing two-step purification system.

Highly purified antigenases were submitted to WST assay to investigate the ability of cell cytotoxicity using A549, SNU-1 (stomach cancer), PANC-1 (pancreas cancer) and BxPC-3 (pancreas cancer) cells. The #1 antigenase showed the cytotoxicity to both A549 and SNU-1 cells but not to PANC-1 cells. The wild type (the dimeric form was rich rather than the monomeric form) showed the stronger effect than the mutant (monomeric form). The order of the strength of the cytotoxicity was #1 WT> #7 WT>#1 MUT> #9 WT>#11WT for A549 cells. That for SNU-1 cells was #1 WT>23D4 WT>#1 MUT. For PANC-1 cells, no antigenase was effective. Proliferation of BxPC-3 was inhibited by #4 WT antigenase. Namely, antigenases which were found in this experiment showed the specificity to the cancer cells.

## Reference

[1] E. Hifumi et al., *Biotechnol Bioeng* **86** (2004), 217–225; T. Uda and E. Hifumi, *J Biosci Bioeng* **97** (2004), 143–152; E. Hifumi et al., *J Biol Chem* **283**(2) (2008), 899–907; E. Hifumi et al., *FEBS J* **277** (2010), 3823–3832.

## Selections with cells and membranes using a yeast-based IgG discovery platform enables isolation and successful optimization of human antibodies against integral membrane proteins

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The success of antibody discovery and optimization programs often depends on the quality of antigen used, and in the case of recombinant or synthetic molecules on their ability to correctly represent the native target. Complex structure and intimate association with lipids creates many technical challenges when working with recombinant forms of integral membrane proteins. This in turn generates a bottleneck in therapeutic antibody discovery against targets like GPCRs, drug transporters and ion channels. In addition, recombinant versions of large extracellular domains of single-pass transmembrane proteins often do not accurately represent relevant epitopes. Adimab has developed a yeast-based antibody discovery platform that delivers therapeutically relevant full-length human IgGs with high affinities, broad epitope coverage and good expressability. By employing intact mammalian cells and membrane preparations we were successful in developing efficient and reproducible methods for the yeast-based discovery and optimization of antibodies against membrane proteins. Two case studies including a GPCR target will be presented.

## Developing the next generation designer cell lines as a customized approach for optimized therapeutic protein production

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The Chinese hamster ovary (CHO) cell line is the predominant host for the production of therapeutic proteins. For the past 10 years, Selexis has developed patented technologies and scale-up processes to produce CHO manufacturing cell lines for clients that routinely achieve expression levels of 1–5 g/L. However, even with these improvements, the CHO cell remains poorly efficient in the production of certain classes of proteins, such as fusion proteins. In order to provide suitable solutions, we have identified components of the secretion network able to enhance the yield of recombinant proteins. We have then developed new designer CHO cell lines that have been metabolically engineered to overcome the specific expression bottlenecks associated with difficult-to-express proteins. These next generation designer cell lines are powerful tools that can be implemented at the earliest stage of discovery up to the product manufacturing allowing considerable labor and time saving.

## Human primary B lymphocytes for the rapid high throughput discovery of native therapeutic monoclonal antibodies

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VIVA|SCREEN is a breakthrough technology that allows the efficient and rapid isolation of highly po-

tent fully human native monoclonal antibodies without being hampered by the antigen-specific but non-biologically active antibodies that often represent over 95% of all antibodies discovered against a defined target.

Three specific achievements were instrumental to the successful development of this technology:

- The access to a very large population of human donors, healthy or diseased, through a network of agreements with blood transfusion centers and hospitals.
- The optimization of the culture and expansion of human primary B cells, to isolate in two weeks populations of B lymphocytes secreting antigen-specific antibodies confirmed to display potent biological activity.
- The use of specifically-designed microarray chips that contain from 62,500 to 234,000 wells with size and shape optimized for a single human B lymphocyte per well, enabling the rapid analysis of the primary B lymphocytes at the single cell level. Using the principle of ELISA, individual B lymphocytes secreting the biologically active antigen-specific antibodies are retrieved, used for RNA isolation, antibody gene cloning and production of recombinant antibodies.

This technology was successfully applied for a series of targets and allowed the cloning of a large number of potent native human antibodies, including antibodies produced by B lymphocytes present only at a very low frequency in human PBMCs ($<$ 1/100,000,000).

## Synthetic human antibodies targeting staphylococcal enterotoxin B (SEB) for prophylaxis and treatment of toxic shock syndrome

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Staphylococcal enterotoxin B (SEB), one of the most potent enterotoxins produced by *Staphylococcus aureus*, is a primary causative agent of toxic shock syndrome (TSS) and food poisoning as well as a potential agent of bioweapon. Currently no therapeutic is available for this toxin. By using phage display technology, synthetic human antibodies against SEB were generated, with several exhibiting low- or sub-nanomolar affinities. Their *in vitro* and *in vivo* toxin neutralizing activity was characterized. The antibodies effectively inhibited T cell responses in cultures of human peripheral blood mononuclear cells (PBMCs). Several lead anti-SEB antibody candidates were tested for their therapeutic efficacy in the mouse toxic shock model. The lead candidates displayed full protection from lethal challenge over a wide range of SEB challenge doses in both prophylactic and one hour post-exposure settings. Furthermore, mice that were treated with anti-SEB IgG had significantly lower IFN$\gamma$ and IL-2 levels in serum compared to mock-treated mice. Our results suggest these anti-SEB monoclonal antibodies may be excellent therapeutic candidates for treatment of SEB-induced disease and lethality.

## Expression and biological characterization of an anti CD20 biosimilar candidate antibody. A case study

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The CD20 molecule is a non-glycosylated protein expressed mainly on the surface of B lymphocytes. In some pathogenic B cells, it shows an increased expression, thus becoming an attractive target for diagnosis and therapy. Rituximab is a chimeric antibody that specifically recognizes the human CD20 molecule. This antibody is indicated for the treatment of non-Hodgkin lymphomas and autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus. In this work, we describe the stable expression and biological evaluation of an anti-CD20 biosimilar antibody. While rituximab is produced in fed-batch culture of recombinant Chinese hamster ovary (CHO) cells, our biosimilar antibody expression process consists of continuous culture of recombinant murine NS0 myeloma cells. The ability of the purified biosimilar antibody to recognize the CD20 molecule on human tumor cell lines, as well as on peripheral blood mononuclear cells from humans and primates, was demonstrated by flow cytometry. The biosimilar antibody induced complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity and apoptosis on human cell lines with high expression of CD20. In addition, this antibody depleted CD20-positive B lymphocytes from peripheral blood in monkeys. These results

indicate that the biological properties of the biosimilar antibody compare favorably with those of the innovator product, and that it should be evaluated in future clinical trials.

## Biological features of human catalytic antibody light chains showing suppressive effect on influenza virus infection

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We have successfully developed some murine catalytic antibodies by the immunization of ground-state peptide or protein into mice to prepare monoclonal antibodies since 1998 [1–3]. For one half of this decade, we are challenging to prepare human catalytic antibody light chain (antigenase), based upon our concept in which subgroup II of human antibody kappa light chain has a high possibility to exhibit an enzymatic function.

Based on the above concept, we amplified and cloned cDNAs encoding the human antibody light chains (kappa) belonging to subgroup II. The obtained cDNA (21 kinds) were transformed into *E.coli* and each human light chain was expressed as the protein, followed by the two-step purification. The highly purified (over 95%) human light chains were submitted to investigate the infection of influenza virus type A using MDCK cells *in vitro* or balb/c mice *in vivo*.

We found some human catalytic light chains capable of suppressing the infection of influenza virus type A *in vitro* assays, where influenza virus H1N1 (A/Hiroshima/37/2001) and H3N2 (A/Hiroshima/71/2001) were used. *In vivo* assay, mice which are capable of infection with the strain PR-8 (H1N1) were employed in this experiment. Out of the investigated antigenases, #4 clone showed the suppressive effect on the infectivity of influenza virus H1N1 not only *in vitro* but also *in vivo* assay. 22F6 clone exhibited a unique characteristic to prevent from the infection of influenza virus H1N1. It seemed that the clone could exert by the different mechanism from that of #4 for the suppression of the infectivity. Interestingly, both #4 and 22F6 antigenases showed the catalytic activity as a DNase to hydrolyze pBR322. Investigation for H3N2 virus was also performed and some antigenases possessed the suppressive effect *in vitro*.

The mechanism how these antigenases were effective to suppress the infection of influenza viruses are under investigation, which will be discussed in the presentation.

### References

[1] T. Uda et al., *J Biosci Bioeng* **97** (2004), 143.
[2] E. Hifumi et al., *J Biol Chem* **283** (2008), 899.
[3] E. Hifumi et al., *FEBS J* **277** (2010), 3823.
[4] E. Hifumi et al., *J Am Chem Soc* **133** (2011), 15015.