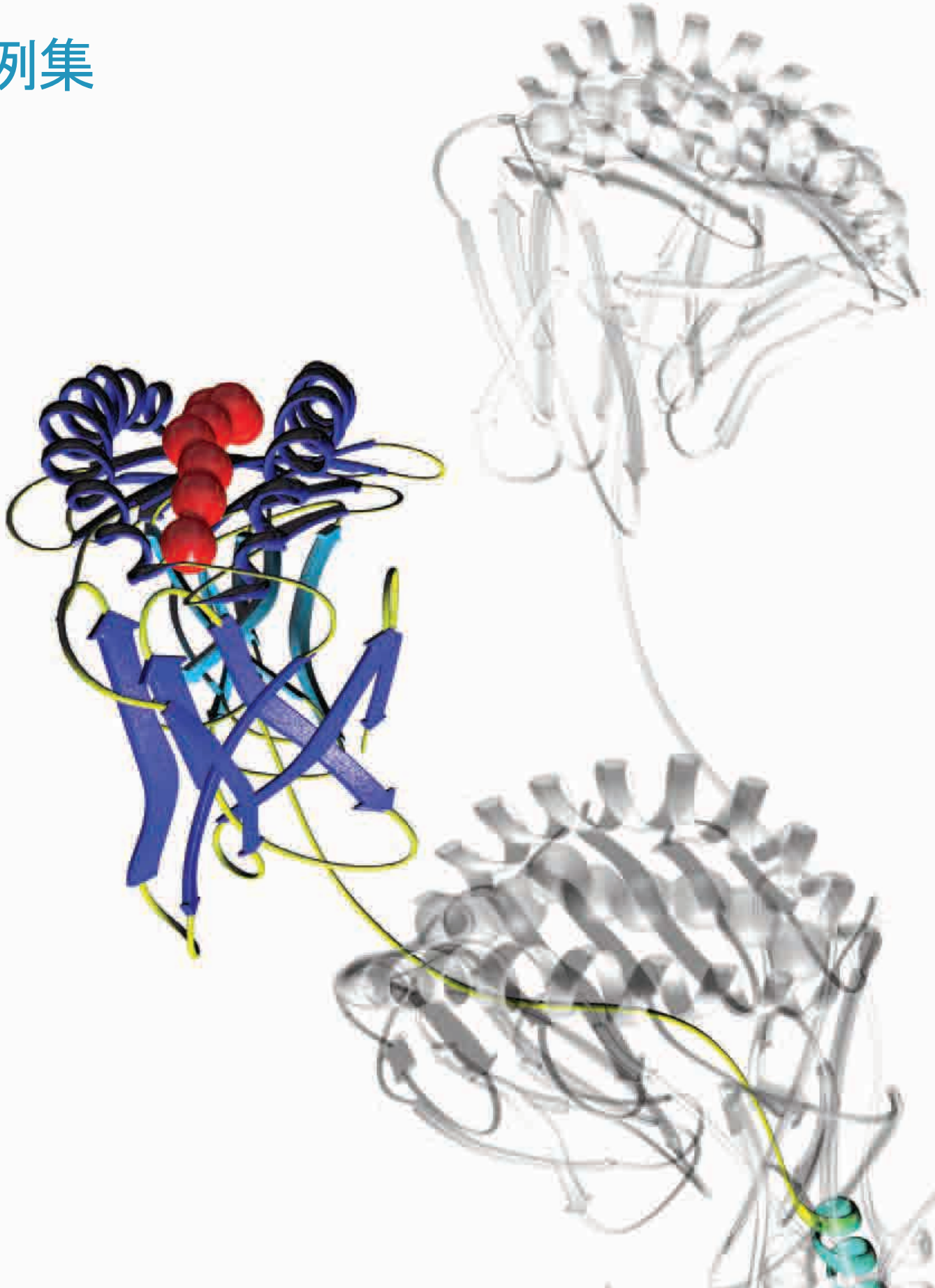
ProImmune

Pro5® MHC クラスI ペンタマー

事例集



CASE STUDY

Pro5® MHC Class I Pentamer combined with intracellular cytokine staining shows how protein translation affects the molecular dynamics of antigen presentation

Tellam et al. (2006) Influence of translational efficiency of homologous viral proteins on the endogenous presentation of CD8+ T cell epitopes. J Exp. Med. 204: 525-32

This study at the Queensland Institute of Medical Research and Harvard Medical School used a Pro5® MHC Class I Pentamer with intracellular cytokine staining to compare the immune responses to EBV encoded nuclear antigen EBNA1 with and without its internal Gly-Ala repeat sequence (EBNA1 and EBNA1-GA respectively). These two versions of the protein have significantly different protein translation efficiencies. A combination of the B*08:01/FLRGRAYGL Pro5® Pentamer and co-staining for interferon gamma expression was used to assess the activation of FLR-specific T cells that were stimulated with lymphoblastoid cell lines (LCLs) expressing the two different EBNA1 proteins.

Figure 1

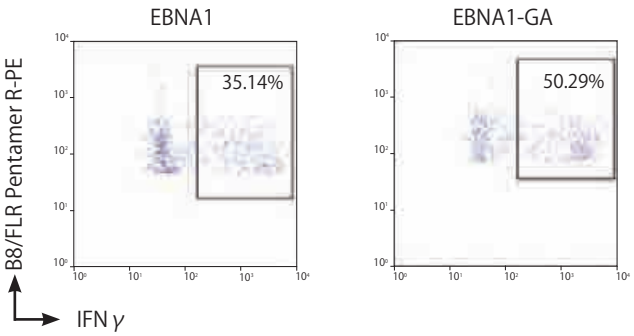


Figure 1: A significant increase in Pentamer-positive and interferon gamma-expressing T cells was observed when EBV EBNA1 specific T cells were stimulated with LCLs that were transduced with an adenoviral construct carrying the EBNA1 antigen without the Gly-Ala repeat (EBNA1-GA) compared to those with the Gly-Ala repeat (EBNA1).

CASE STUDY

Study using HIV-specific Pro5® MHC Class I Pentamers combined with intracellular cytokine staining provides useful data for the develop -ment of T cell vaccines

Almeida et al.(2007) Superior control of HIV-1 replication by CD8 T cells is reflected by their avidity, polyfunctionality, and clonal turnover J Exp. Med. 204: 2473-2485

Almeida et al. carried out an in-depth analysis of the MHC Class I B*27:05/KRWIILGLNK (KK10) HIV-1 epitope. Previous studies have shown that the presence of B27-KK10 epitope-specific T cells confers some protection against progression of the disease in HIV-positive individuals.

Pro5® Pentamers specific to several HIV-1 epitopes were used to identify the antigen-specific cells, which were then further analyzed by flow cytometry to determine their phenotypic characteristics.
Additionally, the capacity of B27-KK10-specific CD8 T cells to produce effector cytokines and to release cytotoxic factors upon antigenicstimulation was assessed with a B*27:05/KRWIILGLNK (KK10)-specific Pentamer and intracellular cytokine staining in order to provide a picture of CD8+ T cell functional quality.

Interestingly, the B27/KK10 CD8+ T cell population showed a significantly higher proportion of cells producing effector cytokines and cytotoxic factors compared with other HIV-specific CD8+ T cells, suggesting that these cells are highly functional. This polyfunctional capability may contribute in part to the protective characteristics and better disease control associated with this epitope. The authors commented that combined data from the study might aid in the development of successful T cell vaccines.

Figure 2

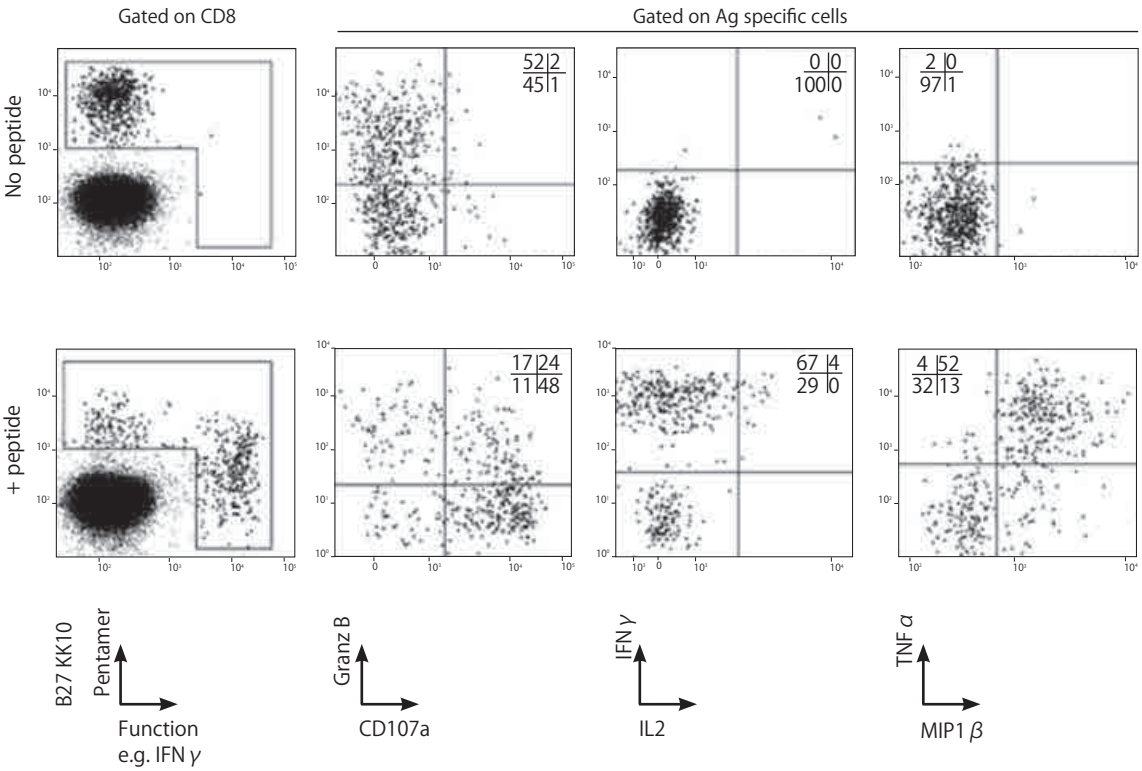


Figure 2: A representative example of simultaneous multifunctional assessment of B27-KK10-specific CD8+ T cells using nine -colour flow cytometry. Cells were stimulated for 6 h in the presence of cognate peptide before intracellular cytokine staining. Percentages of cells in the different quadrants are shown. Plots are gated on CD3 CD8+ cells. Copyright Rockefeller University Press 2007.

CASE STUDY

Pro5® MHC Class I Pentamers used to separate HBV-specific T cells for subsequent phenotypic analysis

Gehring et al. (2007) The level of viral antigen presented by hepatocytes influences CD8 T-cell function. J Virol., 81: 2040-2049

Using virus-specific CD8+ T cell clones and primary human hepatocytes, Gehring et al.analyzed the modulation of CD8+ T cell function following recognition of peptide pulsed or virally infected hepato -cytes. The T cell clones were generated from PBMC from HLA-A2 HBV infected donors by labeling the cells with R-PE-conjugated HBc18-27 specific Pro5® Pentamer and purifying via magnetic cell sorting using anti-R-PE microbeads. Separated cells were cloned by limiting dilution assay and clones were expanded using allogeneic irradiated PBMC feeder cells.

Figure 3

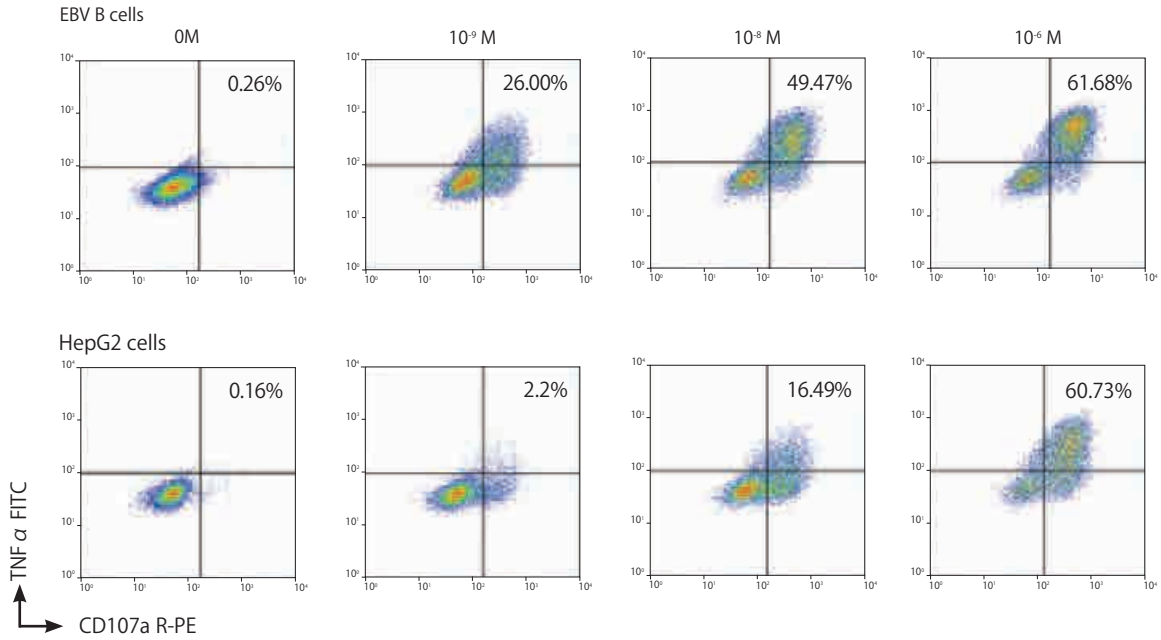


Figure 3: The production of CD107a and TNF-αfrom the HBc-specific T cells (generated through magnetic cell sorting with an HBc -specific Pentamer) was measured after 5 hours of incubation with EBV B cells or HEPG2 cells pulsed with the peptide concentrations shown.

It was observed that limiting the amount of viral antigen in infected human hepatocytes preferentially stimulates CD8+ T cell degranulation, which may lead to hepatocyte damage that is directly caused by virus specific T cells in chronic hepatitis patients.

CASE STUDY

In Situ Identification of Allospecific B Cells using Pentamers

Panoskaltsis-Mortari A., et al.(2008) Blood 111: 3904-3905

Graft rejection following bone marrow transplantation is a serious problem for patients who have undergone this treatment. Amongst other factors, the presence of preformed alloantibody is a major contrbutoributor to the failure of bone marrow engraftment.

A recent study by Panoskaltsis-Mortari et al. demonstrated a novel protocol using ProImmune's Pro5® MHC Class I Pentamers to detect allospecific B cells in situ by immunohistochemistry. Splenocytes from BALB/c mice (H2d) were injected into C57BL/6 mice (H2b) that were then sacrificed after 3 weeks.
Splenic cryosections were stained with an H-2Kd-specific Pentamer (GYKDGNEYI) and, as a negative control, an H-2Dk Pentamer (RRLGRTLLL). BALB/c H-2Kd specific cells were detected in the splenocyte sections, whilst there was no binding of the H-2Dk Pentamer in serial sections. As an additional control C57BL/6 mice were injected with B10.BR splenocytes (H2k). In splenic cryosections the H-2Dk Pentamer showed specific staining, whreas there was no staining with the H-2Kd Pentamer.

Figure 4

3 weeks post-injection of BALB/c splenocytes

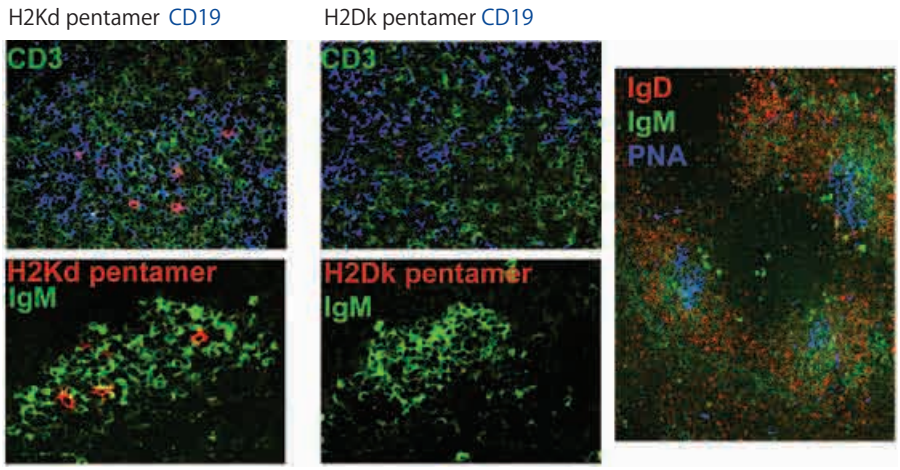


Figure 4 continued

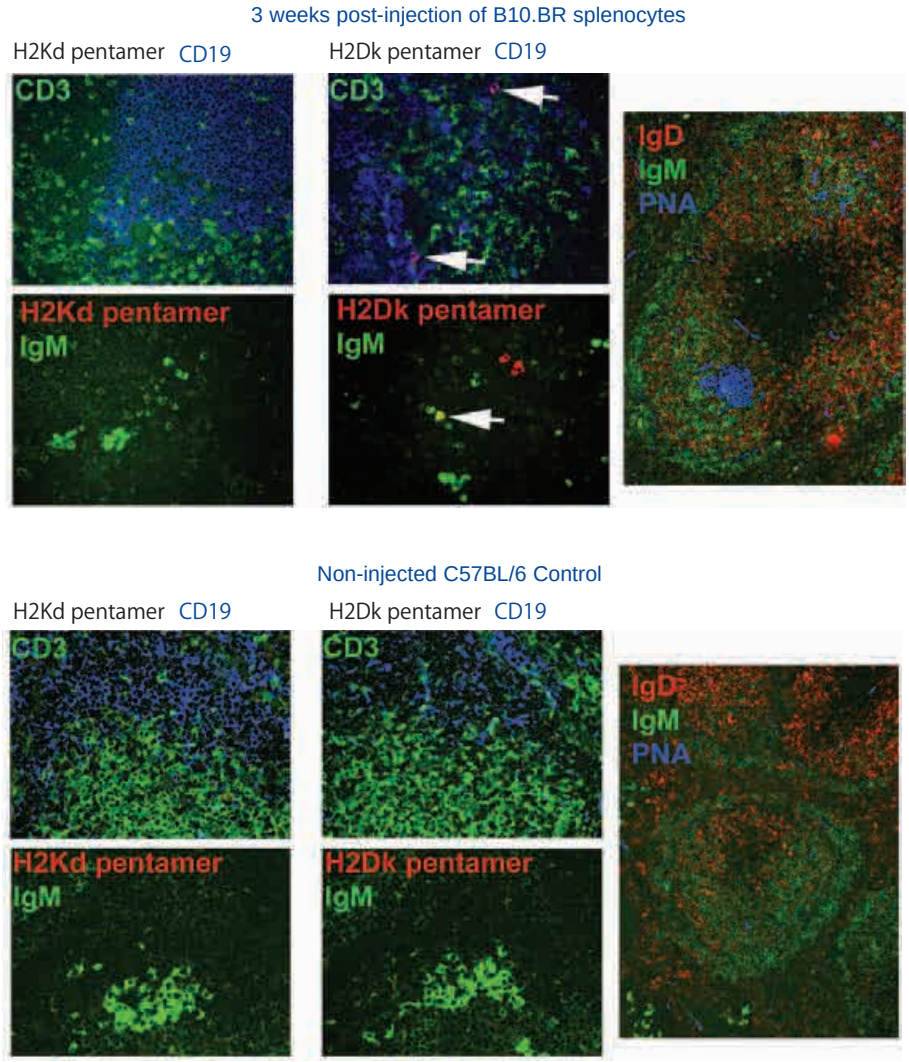


Figure 4: Allospecific B cells can be detected in situ in allosensitized murine spleen using Pro5® Pentamers. Colors for the different markers are indicated in colored text. Magnification is 400x (left and middle panels) and 200x (right panels).

Copyright American Society of Hematology.

This is the first study using Pro5® Pentamers to detect allospecific B cells by immunohistochemistry. This could be an important technique to provide further information about spatial positioning of alloantibody producing cells and inter-cellular interactions. This method could also be used as a way of visualizing antigen specific T cell responses in tissues.

CASE STUDY

CDC study on H5N1 vaccine uses Pro5® Pentamer to correlate immune response with protection

Hoelscher et al. (2006) Development of adenoviral-vector-based pandemic influenza vaccine against antigenically distinct human H5N1 strains in mice. Lancet 367: 475-81

Hoelscher et al. set their objective to develop an egg-independent strategy to combat avian influenza Immunization with human replication incompetent adenoviral based vaccine encoding the hemag -glutinin protein subtype H5 provided effective protection from H5N1 disease, death and primary viral replication in mice. The vaccine induced a three- to eight-fold increase in HA 518-epitope specific T cells secreting interferon gamma. The number of antigen-specific T cells was measured with a Kd/HA 518 -specific custom Pro5® MHC Class I Pentamer to correlate antigen-specific responses with protection.

The result shows the potential of the viral-vector delivery system for an H5N1 vaccine, which is both egg and adjuvant independent and which increases stockpiling options for a pandemic influenza vaccine.

Figure 5

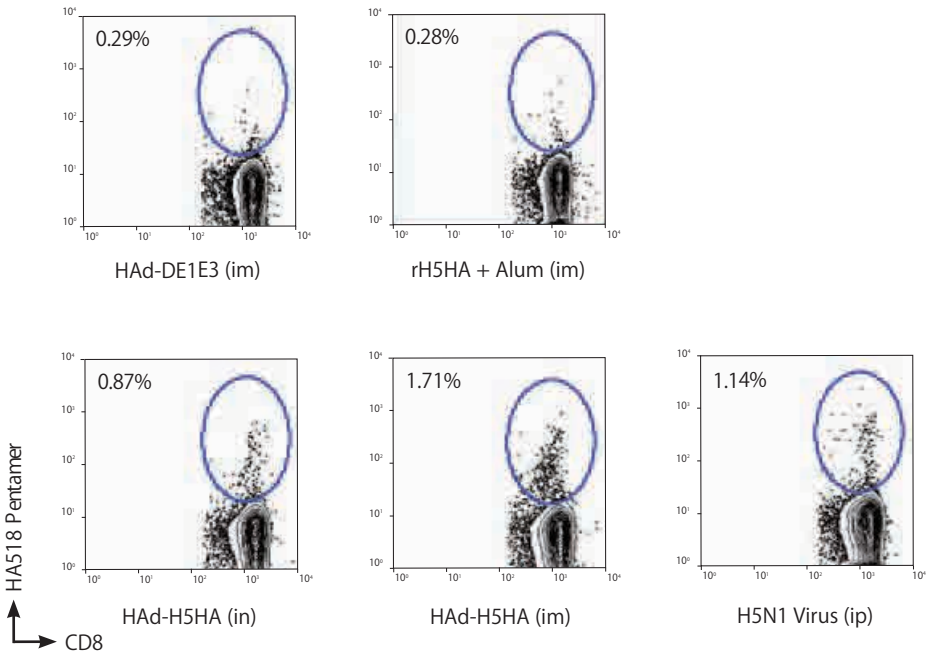


Figure 5: Flow cytometric analysis of spleen cells from immunized mice (three per group) stained with HA 518 Pentamer. Pentamer positive cells (circled) are shown as a percentage of CD8+ T cells. Mice that received the HAd-H5HA vaccine had a three-fold to eight-fold higher frequency of HA 518-specific CD8+ T cells than mice immunized with HAd-DE1E3 or with rH5HA and alum, when delivered intranasally (in) or intramuscularly (im). Mice infected with H5N1 virus showed an HA 518-epitope specific response similar to that recorded with an H1N1 virus infection.

CASE STUDY

Pro5® MHC Class I Pentamers used to evaluate vaccine safety

Elliott et al. (2008). Phase I trial of a CD8+ T-cell peptide epitope-based vaccine for infectious mononucleosis. J Virology 82: 1448-1457

A Phase I clinical trial by Elliott et al.investigated the potential safety of a CD8+ T cell peptide -epitope vaccine against infectious mononucleosis (IM). The vaccine comprised a single EBV EBNA3A derived peptide, FLRGRAYGL, mixed with tetanus toxoid, and was delivered subcutaneously to B*08:01-positive, EBV-seronegative volunteers. EBV-specific Pro5® MHC Class I Pentamers, B*08:01 /FLRGRAYGL and B*08:01/RAKFKQLL, were used to monitor antigen-specific responses post-vaccination in order to ensure that the vaccine was safe in the event of a subsequent EBV infection, and to verify whether or not the immune response was limited to just the single epitope.

Rapid recovery from IM symptoms has been correlated with broad T-cell reactivity to multiple CD8+ T cell epitopes, whereas prolonged illness was associated with a narrowly focused response. Analysis of FLR- and RAK-specific responses in two vaccinees who seroconverted asymptomatically within 2 years of the trial, showed responses similar to those seen in healthy, seropositive individuals or subjects with infectious mono -nucleosis (figure 6).

There was no evidence that vaccinated individuals were predisposed to abnormally high FLR-specific responses after EBV seroconversion, demonstrating that the single epitope vaccine did not lead to an immunodominant response to the single FLRGRAYGL epitope. The study concluded that the single epitope vaccine was well tolerated and immunogenic in subjects, and did not predispose patients to disease following EBV infection.

Figure 6

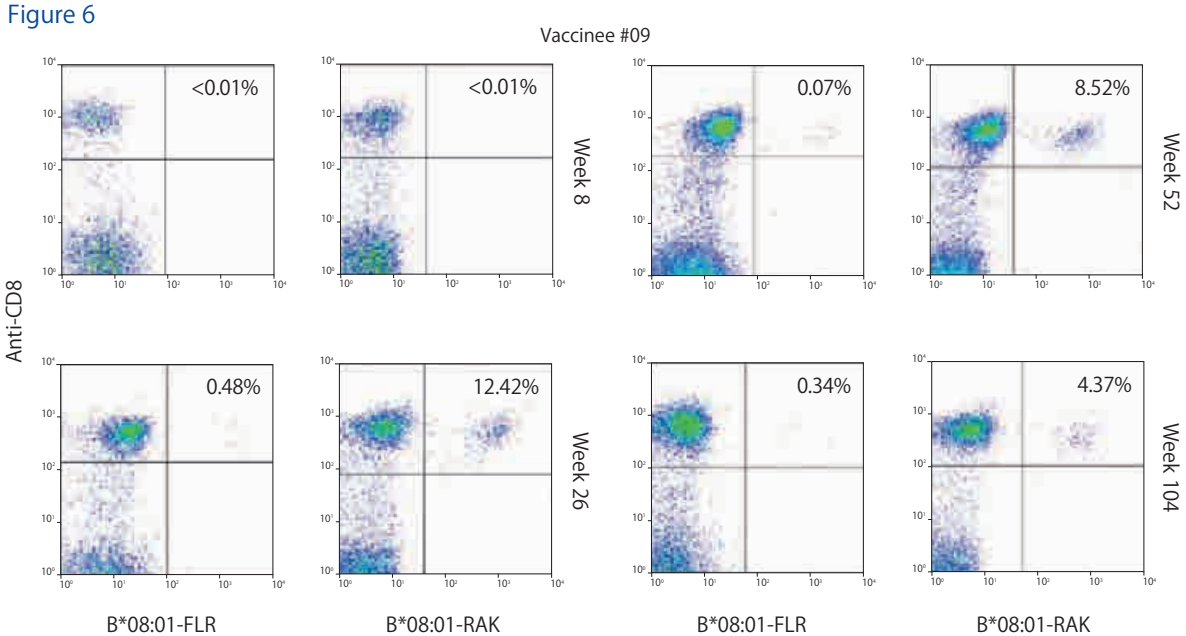


Figure 6: CD8+ T cell response of vaccinees after EBV seroconversion. PBMC from vaccinees (no. 9 and 4) collected at the indicated time points post-vaccination were assessed by flow cytometry using FLR-specific (left) and RAK-specific (right) Pentamers and anti-CD8 monoclonal antibody. The percentage of CD8+ T cells that are Pentamer-positive is indicated in the top right corner of each panel. Copyright 2008 American Society of Microbiology.

CASE STUDY

New adjuvant drives unprecedented cytotoxic T cell response providing a potent vaccine development platform

Wells et al. (2008) Combined triggering of dendritic cell receptors results in synergistic activation and potent cytotoxic immunity. J Immunol. 181: 3422-3431

Wells et al. investigated novel combinations of vaccine adjuvants and, using Pro5® MHC Class I Pentamers, showedthat optimal antigen-specific responses can be achieved using well-tolerated compounds that result in dendritic cell activation through the activation of toll-like receptors.

Initially using the ovalbumin H-2Kb/SIINFEKL epitope as a model, Pro5® Pentamer staining indicated that a vaccine adjuvant referred to as a'combined adjuvant for synergistic activation of cellular immunity' (CASAC) provided the greatest antigen-specific response as indicated by H-2Kb/SIINFEKL Pentamer/CD8+ T cell staining. The vaccination also induced a strong memory response upon re-injection of SIINFEKL peptide as measured by Pro5® Pentamer staining. The CASAC adjuvant contained several key components including two toll-like receptoragonists (e.g. CpG DNA+monophosphoryl lipid A), IFN gamma and CD40 antibody or a class II MHC peptide to induce IL-12 production from dendritic cells. This was combined with SIINFEKL peptide in an emulsion.

The efficacy of the CASAC adjuvant was further tested on a mouse melanoma model using the TRP-2 tumor epitope (H-2Kb/SVYDFFVWL), which is known to bind with a low affinity to the H-2Kb allele. Mice were injected with B16 melanoma cells and then immunized with TRP-2 peptide suspended in either CASAC or the most potent adjuvant combination previously described (anti-CD40 and a single TLR agonist). Pentamer-binding CD8+ cells were only detected in mice that had received the CASAC adjuvant. Protection from tumors was maintained after further challenge with B16 melanoma cells.

The use of Pro5® Pentamers to measure antigen-specific T cell responses accurately enabled the investi -gators to demonstrate that a combinatorial adjuvant approach may provide more effective protection than current anti-cancer vaccine strategies.

Figure 7

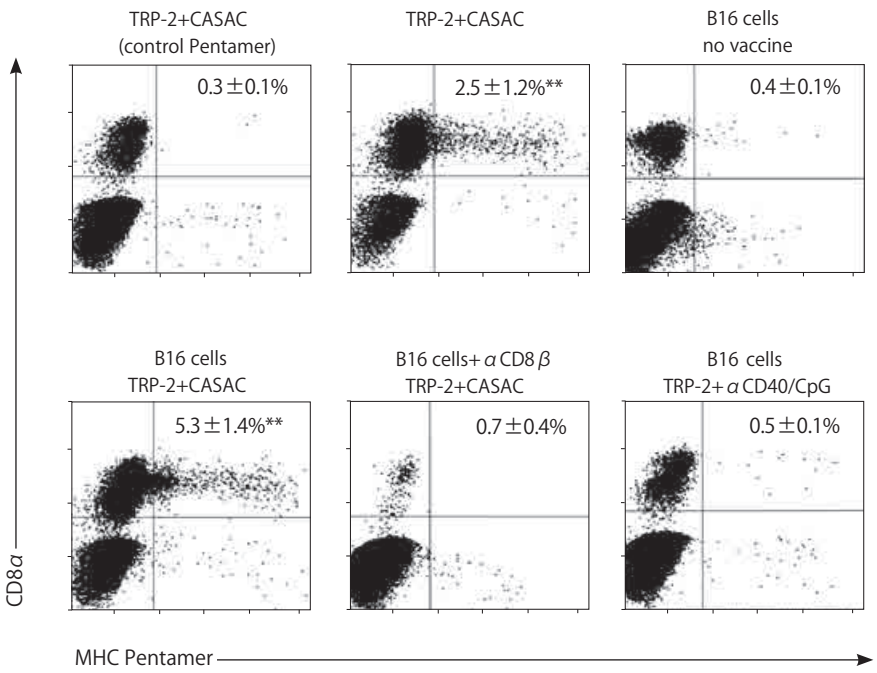


Figure 7: CASAC induces potent tumor protection in a melanoma treatment model mediated partly but not entirely by tumor-Ag specific T cells. The figure shows staining of TRP-2 specific CD8+ T cells in blood of mice using TRP-2(180-188) H-2Kb Pentamer. SIINFEKL Pentamer was used for the negative control stain. Mean percentage ±SEM from 6-8 mice is shown. **p<0.005 as determined by unpaired Student's t tests on the mean values. Copyright 2008 The American Association of Immunologists Inc.

CASE STUDY

Pro5® Pentamer demonstrates effective immunization method for whole protein vaccines

Stoitzner et al. (2008) Tumor Immunotherapy by Epicutaneous Immunization Requires Langerhans Cells. J Immunol. 180: 1991-1998

Immunotherapy using dendritic cells (DC) loaded with tumor antigenic peptides has been shown to lead to improved survival and tumor regression in cancer patients. Epicutaneous immunization through the skin is a new and interesting approach to deliver antigen to resident skin DC. However, the delivery method of these vaccines should be improved to enhance cytotoxic responses.

In this study by Stoitzner et al., epicutaneous immunization was carried out on mice using the ovalbumin (OVA) protein administered in a cream via the ear skin. The immunizations were given in the skin using protocols to induce migration of skin DC. The method of epicutaneous immunization targets epidermal Langerhans cells as well as dermal DC as antigen presenting cells, both of which can present the vaccine effectively to the immune system.

An OVA specific Pro5® MHC Class I Pentamer (H-2Kb/SIINFEKL) was used to detect antigen-specific CD8+ T cell responses by flow cytometry in red-cell lyzed tail vein blood. The levels of CD8+, Pentamer+ cells were increased nearly four-fold in mice immunized through barrier-disrupted skin compared to normal skin and a PBS-immunized control. This T cell response remained higher for up to 21 days after immunization.

Figure 8

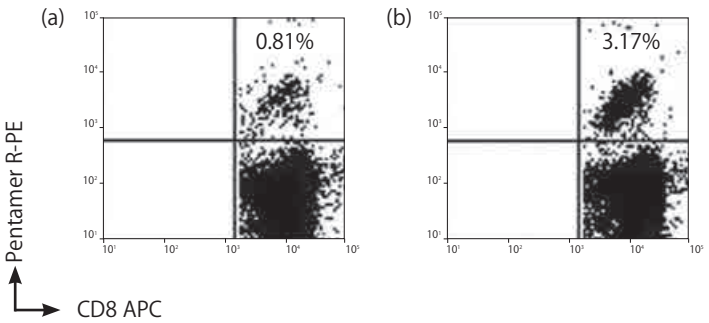


Figure 8: Representative Pentamer staining on day 3 after immunization. The response from the treated skin + OVA group (b) was significantly higher than the group receiving OVA on untreated skin (a). Copyright 2008 The American Association of Immunologists, Inc.

Skin disruption would be an easy and non-invasive method to use in clinical trials. To date this method of delivery has been used mainly with single peptide vaccines. This study highlights that the epicutaneous immunization method can be used with a whole protein antigen. Whole protein vaccines could be given to patients with any HLA allele and could elicit an MHC class II response as well as a class I response.

CASE STUDY

Pentamers identify allo-restricted T cells in study to advance research into cancer immunotherapy

Stronen et al. (2009) Dendritic cells engineered to express defined allo-HLA peptide complexes induce antigen-specific cytotoxic T cells efficiently killing tumor cells. Scand J Immunol. 69: 319-328

Many tumor-associated antigens (TAA) are derived from self proteins that are expressed at low levels in normal tissues. Cancer patients therefore do not produce a cytotoxic T lymphocyte (CTL) response to tumors over-expressing these TAA as they are recognized as self proteins. If CTL can be generated for TAA that are presented by foreign MHC molecules and transferred to the cancer patient, self-tolerance could be avoided. These allo-restricted CD8+ T cells could be used as an immunotherapy to kill the patient cancer.

Stronen et al. investigated the use of dendritic cells (DC) as antigen-presenting cells to generate highly specific, functional allo-restricted T cells. Monocyte derived dendritic cells from HLA-A*02:01 negative individuals were transfected with A*02:01 and loaded with MART-1 (ELAGIGILTV) peptide. The transfected DC were co-cultured with monocyte-depleted PBMC from the A*02:01 negative donor and reactive T cells identified using a Pro5® A*02:01/MART-1 Pentamer. Pentamer-positive cells were sorted by FACS or magnetic bead isolation and then expanded in vitro (figure 9). These MART-1 Pentamer positive T cells effectively killed A*02:01 melanomatumor cell lines indicating that the sorted and expanded cells remain peptide specific.

The validity of this novel methodology was confirmed by generating cell lines for peptides from 2 other leukemia associated self-antigens for CD33 and CD19. Peptides were selected using binding algorithms to predict potential epitopes. Custom Pro5® Pentamers were synthesized for both complexes (A*02:01/$CD33_{9\text{-}17}$ LLWAGALAM andA*02:01/$CD19_{279\text{-}287}$ VLWHWLLRT). Pentamer-positive cells could be detected ex vivo after 19 days in culture andthese cells were successfully expanded whilst retaining peptide specificity against peptide pulsed A2 transfected EBV-LCLs (figure 10).

Figure 9

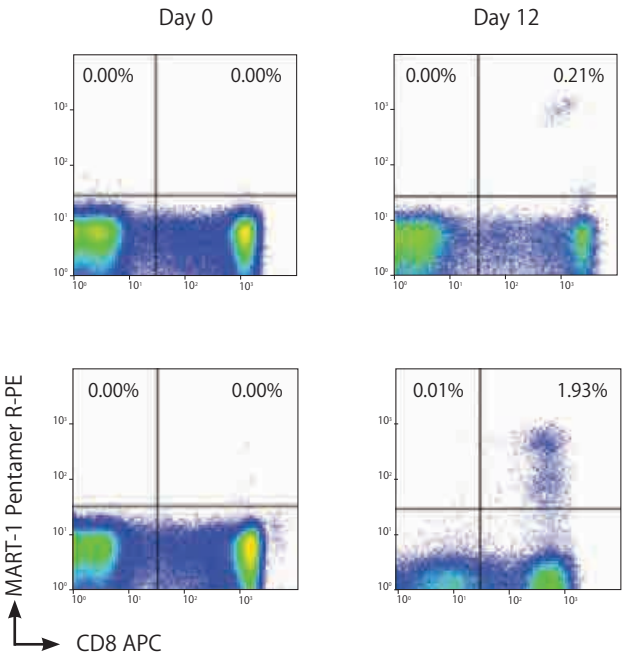


Figure 9: Induction of MART-1-positive cytotoxic T lymphocytes (CTL) from human leukocyteantigen (HLA)-A2-negative donors. Data plots are representative of PBMC from HLA-A*02:01 donors showing anti-CD8 and A2/MART-1 Pentamer immediately after isolation (left) or after 12 days of co-culture with MART-1 peptide-pulsed A2-mono -cyte-derived dendritic cells (right).

Figure 10

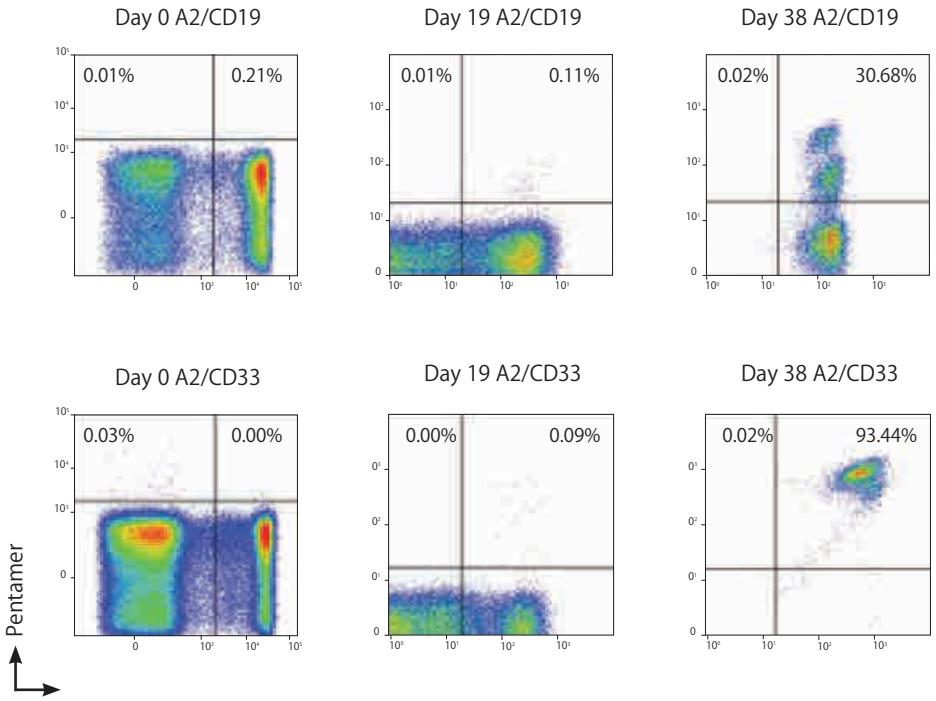


Figure 10: Allo-restricted T cells specific for $CD19_{279-287}$ and $CD33^{9-17}$. Data plots are representative of PBMC from HLA-A*02:01 donors showing anti-CD8 and A2/CD19 or A2/CD33 Pentamer immediately after isolation (left), after 19 days (centre) or 38 days (right) of co-culture with specific peptide-pulsed A2-monocyte-derived dendritic cells.

These data show that it is possible to isolate allo-restricted, antigen-specific T cells and expand these cells to high numbers whilst still retaining functional specificity. The use of both catalog and custom Pro5® Pentamers to identify and confirm specificity of the allo-restricted T cells was essential in this study.

CASE STUDY

Pro5® Pentamers central in life-saving procedure for PTLD treatment

Uhlin et al. (2010) A novel haplo-identical adoptive CTL therapy as a treatment for EBV-associated lymphoma after stem cell transplantation. Cancer Immunol Immunother. 59: 473–477

Uhlin et al.described the first use of Pro5® Pentamers in a rapid method for isolating EBV specific T cells for adoptive cytotoxic T lymphocytes (CTL) transfer.

Three months after receiving a cord blood transplant for acute myeloid leukemia, the patient presented with Epstein-Barr virus (EBV) associated lymphoma, high blood EBV titers and lesions in the lungs, liver, adrenal gland and kidneys indicative of post-transplant lymphoproliferative disorder (PTLD). The patient was unresponsive to standard PTLD treatments and as no other options were available, adoptive EBV -specific CTL transfer from the patient's mother was considered.

Given the life-threatening situation for the patient, a rapid method for CTL preparation was required.
EBV specific T cells (A*02:01/GLCTLVAML) from the maternal blood were stained using Pro5® Pentamers and subsequentlylabeled with magnetic beads allowing a quick and simple isolation of the Pentamer positive cells. The antigen-specific cells were injected intravenously into the patient at a concentration of 1.1x10^{4}CTL/kg.

After only 36 hours, the frequency of Pentamer-positive antigen-specific T cells detected in the patient increased from 0.3% of total CD8+ cells to 4.4%, indicating that the donor cells had expanded in vivo rapidly. Furthermore,following the CTL transfusion, EBV titers decreased back to normal levels almost immediately. By 189 days after CTL transfer, all PTLD lesions had disappeared. At 12 months post-transplant, after being admitted with EBV virus in the tonsils, blood and colon, the patient was given a second infusion, using Pro5 Pentamers to isolate both A*02:01/GLCTLVAML and additional A*02:01/CLGGLLTMV EBV specific cells (2x10^4 CTL/kg). By 72 hours post-infusion, all signs of tonsillitis and enteritis had been cured.

The use of the Pro5® Pentamers was central to developing this novel and successful method for treatment of EBV-associated complications following stem cell transplantation and offers new life-saving strategies for rapid treatment of PTLD patients.

Figure 11

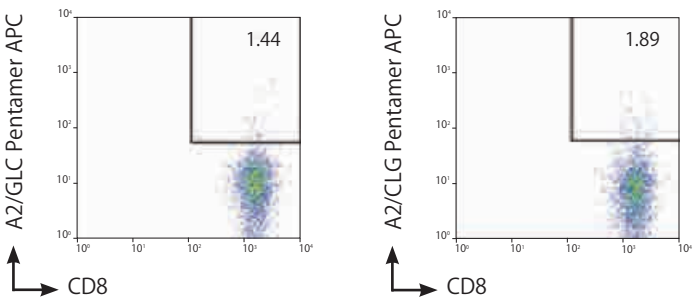


Figure 11: Representative experimental data showing EBV specific cells (A*02:01/GLCTLVAML or A*02:01/CLGGLLTMV) stained with Pro5® MHC Pentamer. These can then be labeled with magnetic beads and separated out for transfer to the recipient.

CASE STUDY

Killer T cells trained for long-lasting anti-cancer effects

Butler M.,et al. (2011) Establishment of antitumor memory in humans using in vitro-educated CD8+ T cells. Science Translational Medicine 3: 80ra34 [PubMed ID: 21525398]

Cancer immunotherapy is an exciting area of research, designed to harness the power of the body's own immune system to fight the disease. For Marcus Butler and his colleagues at the Dana Farber Cancer Institute, the dream would be to generate a library of killer T cells that could be used to target and destroy cancer in patients. Recently published work from this team gives hope that this dream could become a reality in the next 5-10 years following their research on melanoma.

Melanoma is a highly malignant tumor of the pigmented cells found in skin, and in its advanced stage has a poor prognosis - the median patient survival time is less than a year. In melanoma, and other cancers where the tumor antigens are well-characterized, there has been a gurgeoning interest in adoptive T cell transfer as a therapy.With this strategy, CD8+ cytotoxic T cells specific for tumor antigens are isolated (by flow-cytometry sorting the bulk CD8+ population and preferentially expanding the antigen-specific population through peptide stimulation), expanded ex vivo,and then returned to the patient. The tumor -specific T cells traffic to the tumor and destroy target cells, controlling or even eliminating the tumor. Since the T cells are patient-derived, there is minimal toxicity, so the therapy remains a very attractive option. However, the effectiveness of adoptive cell transfer can be improved upon. A major limitation is the life-span of transferred T cells – without repeat rounds of treatment and manipulation of the host immune system such as lymphodepletion, the transferred cells persist for only a week on average, and the therapeutic benefit is short-lived.

Marcus Butler and his co-workers thought laterally about this problem, and their results, published in Science Translational Medicine show that they are on the right track to solving it. For adoptive cell transfer to work successfully, the transferred antigen-specific T cells need to persist for longer.
Their idea was to generate effector memory CD8+ T cells (with a surface marker phenotype CD45RA-, CD45RO+, CD62L+/-), possessing both anti-tumor specificity and the longevity associated with an effector memory phenotype.

The team have previously published data on the generation of artificial antigen presenting cells (aAPC). Using aAPC and IL-15/IL-2 cytokine treatment of tumor specific T cells isolated from patients, they are able to expand a population of antigen-specific effector memory T cells that survive well in vitro. The next step was a proof-of concept study to show that these cells could persist and function in vitro.

They recruited 9 patients with end-stage melanoma, and isolated and expanded CD8+ T cells specific for the melanoma antigen MART-1 (ELAGIGILTV) using their aAPC system. MART-1-specific cells were infused into patients twice, at a 35-day interval, without any other manipulation of host immunity.

Using staining with MART-1 Pro5® MHC Class I Pentamer to monitor responses, increased numbers of MART-1-specific cells were observed at 2 and 3 weeks after treatment, and in 3 patients, increased MART -1 cell numbers were apparent up to a year post-infusion.

Figure 12

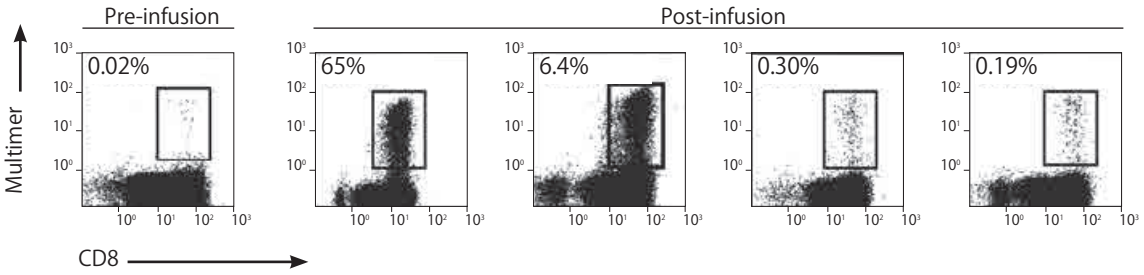


Figure 12: Adoptive transfer induced sustained increases in the frequency of circulating MART1-specific CD8+ T cells. The frequency of MART1-specific T cells was determined by MART1 Pro5® Pentamer staining of circulating CD8+ T cells before and after infusion. RepresentativeA*02:01/ELAGIGILTV Pentamer staining for subject 7 is shown. Day 0 and 35 analyses were performed on blood samples drawn 30 min after infusion of CTL grafts. Data reproduced with permission from Science Translational Medicine.

The transferred cells were followed by monitoring the phenotype of MART-1-specific CD8+ T cells, and could bedistinguished from endogenous MART-1 specific cells through their continued expression of memory markers. Their functionality was confirmed by IFN-gamma ELISpot and also by applying MART-1 peptide to patient to induce a delayed-type hypersensitivity reaction. By both of these measures, anti-MART-1 T cell activity was enhanced after treatment. Importantly, the infused T cells were able to traffic to tumor sites; this was demonstrated by tumor biopsy (an increase in tumor infiltrating lymphocytes was apparent) and by sequencing the T cell receptors of the tumor-infilt -rating lymphocytes and comparing these to the sequences known to be present in the infused cells. 70 days after treatment, one patient had achieved complete remission, four were stabilized, three had progressive disease, and one died before receiving a second infusion of cells. From such a small patient cohort, it is not possible to draw firm conclusions, but the results are very promising.

Following the adoptive cell therapy trial, five patients subsequently received Ipilimumab® as a CTLA-4 blockade. The treatment ameliorated the condition of most of those who received it, and one patient achieved almost complete resolution of her disease. This is of note because the response rate for Ipilimumab® treatment overall is low, at around 16%, and there exists evidence that CTLA-4 blockade can induce expansion of adoptively transferred CD8+ T cells. Butler et al. suggest that the efficacy of their aAPC-induced memory effector anti-tumor cell therapy would be further enhanced by including CTLA-4 blockade in a treatment regime. Combining their enhanced adoptive cell transfer protocol with existing treatments like this could, the investigators suggest, substantially improve prognosis for those who are seriously ill with melanoma.

CASE STUDY

Success for Hepatitis C vaccine trials at Oxford University

Barnes E.,et al. (2012) Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. Science Translational Medicine 4: 115ra1. [PubMed ID: 22218690]

Infection with Hepatitis C virus (HCV) can cause debilitating liver disease, and up to 170 million people worldwide carry the virus. Infected individuals will often be asymptomatic for years, so rather than waiting for HCV patients to present with liver disease, researchers are trying to develop a preventative vaccine.

In a recent edition of Science Translational Medicine, Oxford University researchers reported the first clinical trial of a preventative vaccine for HCV based on T cell activation. Ellie Barnes, Paul Klenerman and their colleagues tested a vaccine designed to elicit a long-lived anti-HCV response from CD4+ and CD8+ T cells.

They worked with adenovirus to provide a sturdy vector for expressing portions of viral proteins. However, most people have at one stage or another been exposed to adenovirus, and will clear the virus before it can start to be effective as a vaccine. To overcome this hurdle, the team developed two different adenoviral vectors based on a rare human adeonovirus (Ad6) and a chimpanzee adenovirus (ChAd3).

It was important to avoid parts of the virus that mutate quickly, such as the viral coat, so Barnes et al. used targets from the viral innards: non-structural proteins 3, 4 and 5. Regions ('epitopes') from these proteins had been characterized as important in immune responses from individuals who had managed to spontaneously clear HCV infection.

The responses from vaccinated individuals were very closely monitored. One aspect of this was monitoring the exact viral epitopes being targeted by the two vaccines. Over the course of the study (up to 52 weeks) the percentage of circulating CD8+ T cells staining with ProImmune's Pro5® MHC Class I A*02:01/KSALGINAV or A*01:01/ATDALMTGY HCV NS3 Pentamers in each individual were tracked.

The data accumulated clearly showed that CD8+ T cell response went from barely detectable to a sustained 1% of the CD8T+ cell population, an impressive result.

Figure 13

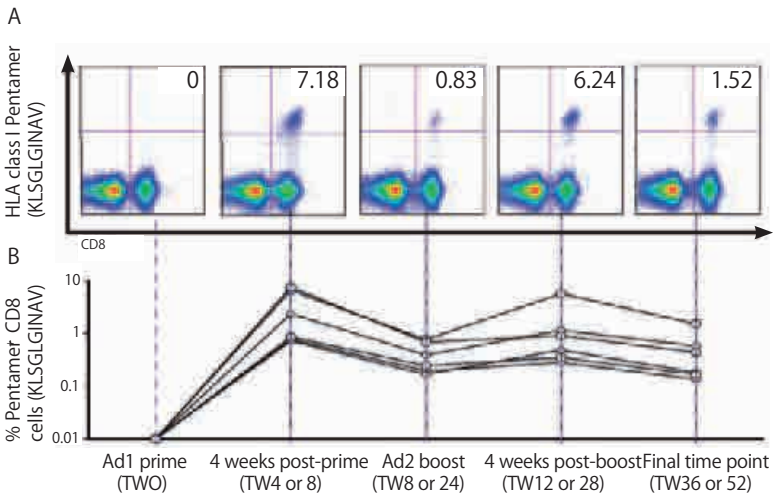


Fig 13: Characterization of epitope-specific T cell responses induced after vaccination with either of the the two test vaccines. (A) Staining with A*02:01/KLSGLGINAV Pro5® Pentamer in a representative subject over the study time course. Gating is on live CD3+T cells. Percentage Pentamer+/CD8+ T cells are shown. (B) *Ex vivo* tetramer+ CD8+ T cell responses over time in six volunteers who responded to the vaccination. Figure reproduced with permission from Science Translational Medicine.

Overall, the anti-HCV responses induced in the trial subjects were prolonged, and broad in terms of the epitopes they targeted. This is important because it should allow the vaccine to work against different HCV strains, and to remain effective even if some of the targets mutate over time.

Speaking to BBC News just after the work was published, Paul Klenerman was optimistic about the promise of his results, stating "The immune responses we've seen are exciting. While we are hopeful, it could be a long road to any vaccine that protects people against hepatitis C".

CASE STUDY

Pro5® Pentamers and viral escape in chronic Hepatitis C virus infection

Kasprowicz V., et al.. (2010) Hepatitis C virus (HCV) sequence variation induces an HCV-specific T-cell phenotype analogous to spontaneous resolution. J Virology 84: 1656-63. [PubMed ID: 19906915]

Hepatitis C virus (HCV) remains today a major global health concern. There is no preventative vaccine, and the majority of infected individuals (some 3% of the world's population) go on to become carriers, which entails long-term health risks. CD8+ T cells (CTL) from patients target the disease, but in a battle with the virus, they often lose.

Kasprowicz et al. used ProImmune's Hepatitis C Virus (HCV) Pro5® Pentamers to investigate the HCV -specific CTL persisting in individuals with chronic infection. These cells are low frequency, which might be expected, but more surprisingly, they exhibit a phenotype more commonly associated with resolved infections, expressing the memory marker CD127.

The team were able to resolve this apparent discrepancy, demonstrating that the CD127low, virus-specific CTL were not specific for the HCV variants circulating in infected individuals. CD127, part of the IL-7 receptor, is normally only expressed on CD8+ T cells specific for antigens from controlled infection; thus CD127 expression is usually low on CTL specific for persisting viruses and high on CTL specific for acute resolved infections such as influenza.

Virus-specific responses in cohorts of patients with acute, chronic or resolved HCV infections were compared. In chronically infected individuals a range of levels of CD127 expression on virus-specific CTL was visible, while as would be expected, all HCV-specific CTL in the resolved patients were CD127high (figure 14A).

The team explored this observation further, since HCV has the potential to escape a CTL response through mutation. Sequencing of the virus in chronically infected individuals demonstrated an interesting correlation. While there was a perfect match between T cell epitope and autologous virus in chronically infected patients with low CD127 expression (figure 14B), those patients expressing higher levels of CD127 did so only on cells that were not specific for circulating virus (figure 14C): there remains an absence of CD127high CTL specific for autologous virus in these individuals, explaining the persistence of HCV infection.

Figure 14

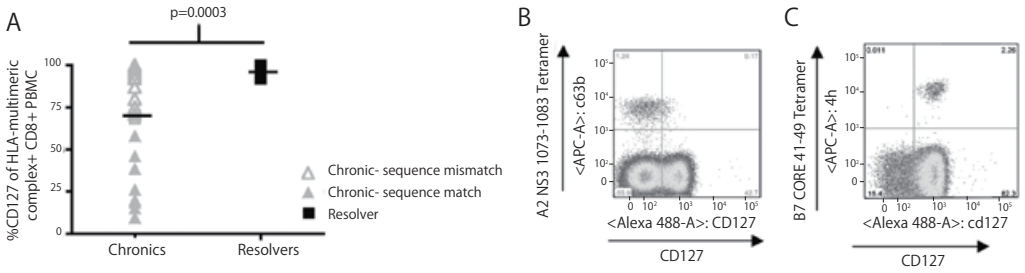


Figure 14: Chronically infected individuals express a range of CD127 levels on HCV-specific T cells. (A) CD127 expression levels on HCV-specific T-cell populations in individuals with established chronic or resolved infection. While individuals with resolved infection uniformly express high levels of CD127, chronically infected individuals exhibit a wide range of CD127 expression levels. (B) Low CD127 levels are observed on HCVNS31073-1081 (CINGVCWTV) -specific T cells from an individual with chronic HCV infection and an intact autologous sequence. (C) CD127 expression levels on HCVcore41-49 (GPRLGVRAT) -specific T cells from an individual with resolved HCV infection. Figure reproduced (with amendments) with permission from the American Society for Microbiology

Using genotype-specific T cell lines, and ELISpot assay, it has previously been shown that expression of CD127 in the presence of viral infection is closely associated with the capacity of the patient's CTL to recognize virus. This was borne out by the case of one patient participating in this study; antiviral therapy led to the loss of CD127negative HCV specific CTL and the emergence of a CD127 population.

By following the progression of HCV infections over a period of months, using Pro5® Pentamer staining to track CTL phenotype, the team were able to conclude that CD127 expression is dependent on the absence of ongoing antigenic stimulation (due to either viral clearance or viral variation).

During the early stages of an acute infection, CD127 expression is low or absent, and increases during the course of a response. Re-encounter with antigen led to an expansion of naïve, CD127low cells in these patients.

Taken together, the data suggest that CTL responses in chronically HCV infected individuals exist but fail to target the circulating virus, so enabling the infection to persist. This observation extends our ability to target persisting infections, looking beyond CTL we now know to be ineffective against circulating virus, and allowing us to focus on stimulating useful CD127low CTL to eliminate the disease.

CASE STUDY

Pro5® MHC Pentamers are used to identify a new target for EBV treatment

Fox CP,et al. (2010) A novel latent membrane 2 transcript expressed in Epstein-Barr virus-positive NK- and T-cell lymphoprol-iferative disease encodes a target for cellular immunotherapy. Blood 116: 3695-704. [PubMed ID: 20671118]

In a recent issue of Blood, Christopher Fox and his collaborators explain a mystery from the EBV field.

Natural Killer/T cell lymphomas are frequently associated with EBV and respond poorly to current therapeutic regimes. The EBV genome encodes some 80 proteins, but very few of these are expressed in tumours. Among these few is the membrane protein LMP2: LMP2 is targeted by CD8+ T cells (CTLs) and shows some promise as anavenue of attack for anti-tumour therapy.

Foxet al. characterized four EBV tumour cell lines, and key to their analysis was the range of LMP2-specific MHC Class I Pro5® Pentamers available from ProImmune, which they used in flow cytometry. They found that LMP2Awas barely detectable as a protein (using an antibody specific for the unique N-terminal of the 'A' variant), and using primers from the 5' end of each transcript found vanishingly small levels of each. Given that anti-LMP2 CTL responses have been previously characterized, this result was baffling.

Importantly, antigen presentation by the cell lines was intact. The team made two different polyclonal anti-LMP2 CTL presentations by stimulating EBV patient PBMC with LMP2-transduced antigen presenting cells.The polyclonal CTLs were active against tumour cell lines, as measured by 51-Cr release assay. Their CTL mixture contained substantial numbers of LMP2-specific cells, identified by Pro5® Pentamer staining (figure 15).

Figure 15

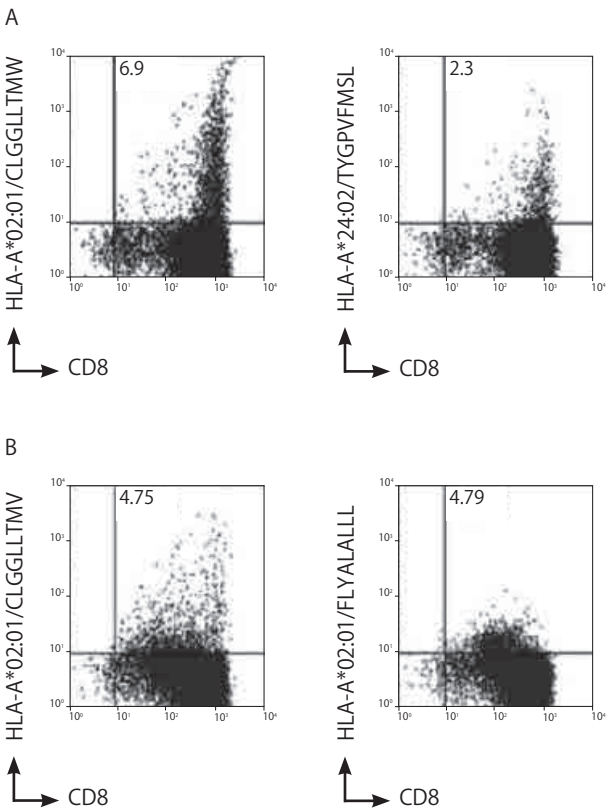


Figure 15: Dual-color flow cytometry of CTLs derived from two EBV-associated lymphoma patients (A and B) prepared by in vivo stimulation with LMP2/LMP1 transfected antigen-presenting cells. CTLs were stained with anti-CD8 antibody and with HLA-peptide Pentamers: HLA-A*02:01/CLGGLLTMV, HLA-A*02:01/FLYALALLL, HLA-A*24:02/TYGPVFMSL as indicated. Numbers in the top right quadrant of each plot indicate the proportion of viable peptide/Pentamer-specific CD8+ cells as a percentage of viable cells. Data reproduced with permission of American Society of Hematology (ASH) via Copyright Clearance Center.

Since activity against other EBV epitopes was not excluded by this analysis, A*24:02 and A*02:01 epitope -specific clonal CTL lines were also tested, and gave comparable results. Widening the HLA scope of their investigation, Fox et al.. transduced one of their tumour cell lines with recombinant HLA-A*11:01, and observed IFN-gamma production from a HLA-A*11:01-restricted LMP2 epitope-specific CTL clone.

So, with anti-LMP2 CTL responses in the context of three different HLA types, in the apparent absence of LMP2 expression – could there be more transcripts from the LMP2 locus? qRT-PCR of 3' exons of the LMP2 locus showed robust expression of a transcript encoding (at least) exons 2-6.
Via 5'-RACE, the new transcripts were found to initiate in the terminal repeat (TR) region of the LMP2 gene, and all four EBV tumour cell lines tested expressed transcripts initiating in the TR.

This result was validated using mRNA extracted from patient biopsies. Of seven EBV-associated lymphomas examined, all expressed the newly-identified transcript. The implications of this finding are far-reaching, as the so-called TR-LMP2 protein, being the only LMP2 variant expressed during NK and T-cell lymphomagenesis, could become a target for future therapies. The ability to map individual epitope responses from CTL, using ProImmune Pentamers, was critical to the success of this work.

This work was carried out in the Tumour Immunology and Gene Therapy labs in the University of Birmingham School of Cancer Sciences (UK), in collaboration with the Bollard laboratory at Baylor College of Medicine, Houston, Texas.

CASE STUDY

Instituto Gulbekian de Ciência researchers show how inheritance of Sickle Cell Anemia protects against Malaria infection

Ferreira A., et al. (2011) Sickle hemoglobin confers tolerance to Plasmodium infection. Cell 145: 398-409. [PubMed ID: 21529713]

Malaria claims the life of a child every 45 seconds, but without the prevalence of sickle cell anemia in populations of sub-Saharan African descent, it would be even more lethal.

Sickle cell anemia is caused by a point mutation in the human hemoglobin (Hb) gene which leads to aggre -gation of Hb and characteristic “sickling” red cell morphology. While sickle cell disease is itself a serious and life-limiting condition, the sickle cell trait persists because inheritance of a single copy (each individual carries two) of mutant Hb is protective against malaria.

Since this survival advantage was first observed, its mechanism has remained elusive – a puzzle known to all high-school biology students.

Now, using ProImmune’s Pro5 MHC class I Pentamers for immune monitoring, a team at the Instituto Gulbenkian de Ciência in Portugal have shown exactly how sickle Hb (HbS) induces tolerance to malaria.

Malaria is caused by infection with Plasmodium parasites. In their study, Ferriera et al. showed that the presence of HbS does not reduce parasite load, but instead impairs the development of malaria disease pathology via two distinct mechanisms.

The team established a mouse model for sickle cell disease and malaria: briefly, mice engineered to express human Hb or HbS were infected with an engineered variant of Plasmodium berghei, and scored for the development of experimental cerebral malaria (ECM). Their transgenic P. berghei was designed to express an MHC Class I epitope from herpes simplex virus glycoprotein B (SSIEFARL),and using ProImmune’s H-2Kb/ SSIEFARL Pro5Pentamer, they were able to monitor pathogen-specific CD8+ T cell numbers accura -tely in experimental mice. The HbS mice fail to succumb to ECM after infection. Compared with wild-type mice, malaria-infected HbS mice showed decreased ECM pathology, including brain edema, and decreased brain accumulation of the CD8implicated in ECM pathology (figure 16).

Figure 16

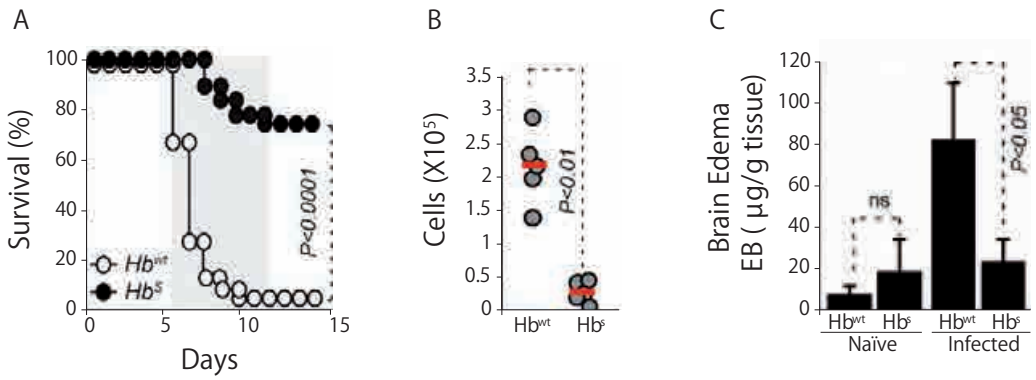


Figure 16. HbS Prevents ECM Onset. (A)Survival of Plasmodium infected Hbwt (n = 91) and HbS (n = 76) mice (10 independent experiments with survival advantage $p < 0.05$). Grey shading: expected time of ECM. HbS mice have a clear survival advantage. (B) Brain malaria-specific (H-2Kb/SSIEFARL Pentamer) CD8+ T cell numbers infected Hbwt and HbS mice, 5 days after Plasmodium infection. Dots represent single mice (n = 4 -14/group). Red lines represent mean values. There are lower numbers of brain-infiltrating malaria-specific T cells in the HbS mice. (C) Mean brain edema in naïve versus infected Hbwt and HbS mice ± standard deviation (n=4/group), 5 days after Plasmodium infection. Hbwt mice have significantly more edema than HbS mice. ns: not significant. Figure reproduced with permission from Elsevier

ECM pathology has previously been attributed to cytotoxic accumulation of free heme. Via a series of mouse crosses, Ferriera et al. were able to establish how HbS counteracts heme accumulation. HbS induces expression of heme-oxygenase-1 (HO-1) in hematopietic cells. HO-1 –deficient mice, or mice in which HO-1 is pharmacologically inhibited, were no longer protected from ECM even when they expressed HbS.
Mice deficient in the transcription factor Nrf2 were used to show that HO-1 expression relies upon Nrf2 expression. The team suggest that HO-1 exerts its protective effect via carbon monoxide (CO). CO is a by-product of heme catabolism by HO-1, and binds free Hb to inhibit further heme release.

Independently of its effects on HO-1, HbS affects the activation and expansion of malaria-specific CD8+ T cells. Using H-2Kb/ SSIEFARL Pentamer staining to compare Hb and HbS-expressing mice five days after infection, they found that there was no expansion of antigen-specific CD8+ T cells in the HbS mice, and that this lack of expansion was still evident when the infected mice were also HO-1-deficient.
While this suggested that the T cell element of malaria pathology is independent of HO-1, there is still a role for heme itself in modulating T cell numbers. Administration of free heme to mice prior to infection was sufficient to limit epitope-specific T cell expansion.

The insights into malaria pathogenesis that Ferriera et al. have gained using ProImmune Pentamers open up this longstanding mystery to further research efforts. Over the next few years we hope to see novel malaria treatments being developed to exploit this new knowledge of both the HO-1 dependent and the immuno -pathologic elements of malaria progression.

CASE STUDY

Epitope discovery in prostate cancer – the use of a custom Pro5® Class I Pentamer in the characterization of an immunodominant CTL epitope of PSA in mice.

Pavlenko M., et al. (2005) Identification of an immunodominant H-2Db-restricted CTL epitope of human PSA. Prostate 64: 50-59

Prostate cancer is a serious condition affecting 1 in 6 men. Prostate specific antigen (PSA) expression is increased in prostate cancer and so is exploited not only for diagnosing and monitoring prostate cancer, but also as a potential target for immunotherapy. By working with Custom Pro5® Pentamers from ProImmune, Pavlenko et al.identified and validated an immunodominant cytotoxic T lymphocyte (CTL) epitope of PSA in C57BL/6 mice. A combined bioinformatics approach using the SYFPEITHI prediction algorithm (www.syfpeithi.com), and biochemical MHC-peptide stabilization assays was used to define the the candidate epitope (H-2Db/HCIRNKSVI) and a custom Pro5® Pentamer was synthesized.

PSA-specific CTLs were induced by immunizing mice with a plasmid expressing PSA (pVax-PSA). Using intracellular cytokine staining as a functional assay and Pro5® Pentamer staining to detect PSA -specific CD8T cells, the authors were able to demonstrate correlating frequencies of IFN-gamma -positive and Pentamer–positive T cells following in vitro stimulation with the specific peptide.

The authors concluded, “Pentamer technology enables detection of TCR-specific T cell populations and, when combined with functional assays, allows the discrimination between anergy and tolerance induction during effector responses. H-2Db Pentamers assembled with this peptide are an efficient tool for monitoring PSA-specific CTL responses after DNA vaccination”.

Figure 17

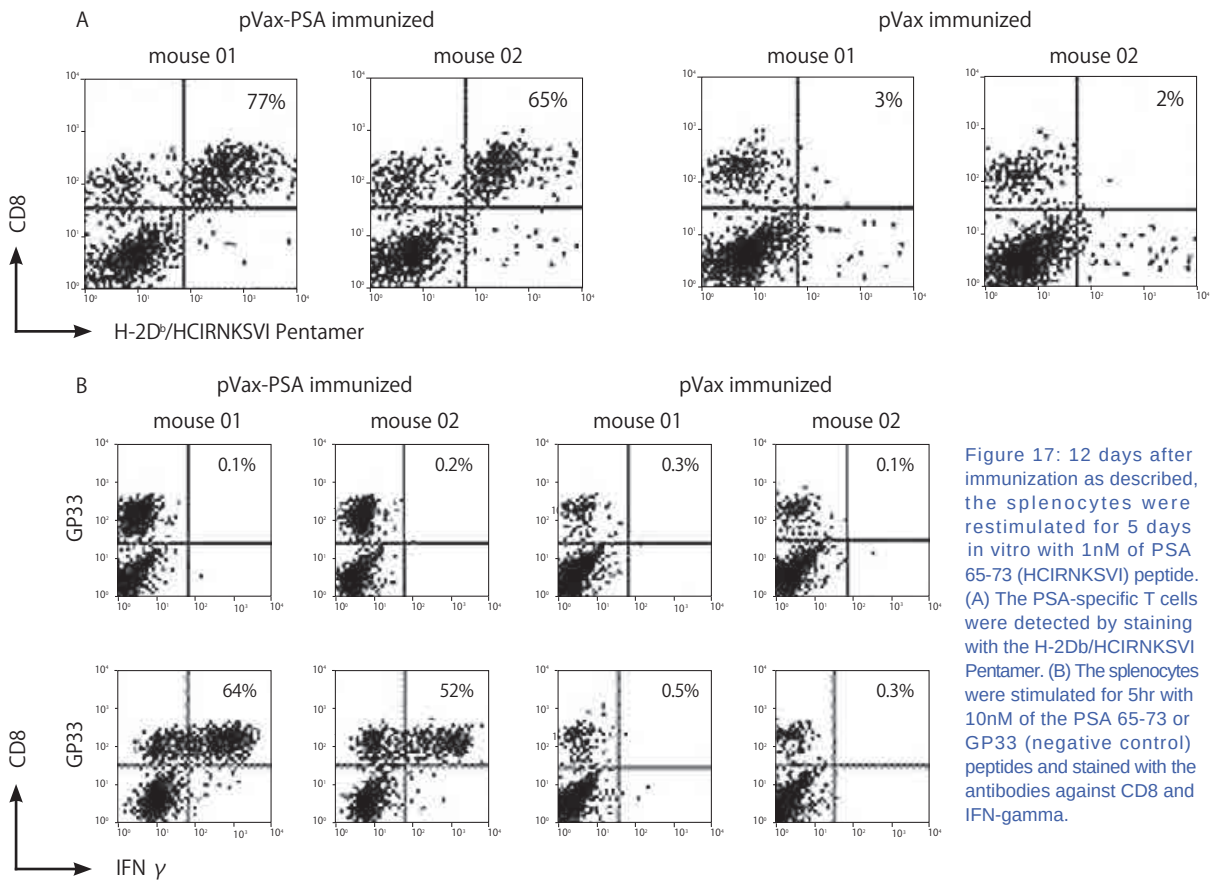


Figure 17: 12 days after immunization as described, the splenocytes were restimulated for 5 days in vitro with 1nM of PSA 65-73 (HCIRNKSVI) peptide. (A) The PSA-specific T cells were detected by staining with the H-2Db/HCIRNKSVI Pentamer. (B) The splenocytes were stimulated for 5hr with 10nM of the PSA 65-73 or GP33 (negative control) peptides and stained with the antibodies against CD8 and IFN-gamma.

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