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MEETING REPORT

Report on the Fifth Annual Meeting of the Association for Immunotherapy of Cancer (CIMT) April 12–14, 2007 in Würzburg, Germany

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Abbreviations

(m)Ab	(Monoclonal) antibody
CTL	Cytotoxic T lymphocyte
DC	Dendritic cell
MDSC	Myeloid derived suppressor cells
s.c.	Subcutaneous(ly)
TCR	T cell receptor
TEC	Thymic epithelial cell
Treg	Regulatory T cell

Introduction

Here we report from the Fifth Annual Meeting of the Association for Immunotherapy of Cancer (CIMT) that was organized as a joint meeting together with the Third International Conference "Strategies for Immune Therapy". Approximately 250 participants came together for 3 days in Würzburg for this meeting, which started at 12 April 2007, to learn more and discuss about recent developments in the field of infectious diseases and cancer immunology. The meeting encompassed three days with six scientific sessions, guided poster sessions that were based on 62 abstracts and a satellite-workshop that was dedicated to immunomonitoring. This report will focus on selected lectures and poster presentations directly related to tumor therapy.

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Development of immunity

B. Kyewski (Heidelberg, Germany) gave a detailed lecture on the mechanisms by which tolerance against self-antigens is generated and maintained. Here, the thymus with its different compartments and cell subpopulations plays the central role. Especially the medullary thymic epithelial cells (mTECs) have been shown to express an enormous array of antigens that were originally thought to be exclusively expressed in peripheral tissues up until some years ago. The mTECs can be further divided into immature and mature TECs, which can be distinguished by the differential expression of the CD80 molecule. The $CD80^{high}$ mTECs constitute the most differentiated subset, present the largest repertoire of MHC-ligands to developing T cells and are also able to transfer antigens to thymic dendritic cells (DCs) for cross-presentation. Both the thymic DCs as well as the mTECSs are short-lived cell populations and as such make the medulla a highly dynamic compartment in which T cells continuously encounter a wide spectrum of antigens. These two cell populations complement each other by deletion of potentially autoreactive T cells (when antigen is presented by thymic DCs) as well as by the induction of antigen-specific regulatory T cells (when antigen is presented by mTECs). The pool of genes that are promiscuously expressed in the mTECs represents most, if not all, peripheral tissues and is estimated to include about 3,000 of all currently known genes. Two models have been proposed

to explain the cell-type specific pattern of promiscuous gene expression. (1) The progressive restriction model, in which immature mTECs mimic gene expression programs of distinct tissue-specific cell lineages and preserve cell-type specific gene regulation, and (2) the alternative terminal differentiation model which proposes that a random pattern of promiscuous gene expression is an autonomous property of mature mTECs. Currently available data tend to support the model of terminal differentiation. Elucidating the mechanisms that balance the induction of tolerance in the thymus will allow a better understanding and subsequently better treatment of autoimmune diseases in the future. The high relevance of only subtle changes in expression levels of autoantigens in mTECs were shown for patients with Myasthenia gravis, in which a single nucleotide polymorphism in the promotor of the α-acetylcholin receptor leads to small changes in the thymic expression level of this gene; these different expression levels clearly correlate with the age of disease onset. Next to deleterious effects of defective central tolerance in case of autoimmune diseases, incomplete or leaky tolerance due to differences in antigen expression in the thymus versus tumor cells (e.g., glycosylation of the tumor-specific MUC1 protein) could be exploited in cancer immunotherapy.

Further insight into the lymphoid organs that regulate the development and selection of lymphocytes was provided by T. Boehm (Freiburg, Germany). Different animal species have different immune systems and it is now clear that even arthropods can generate a diversity of antigen receptors via alternative splicing whereas early agnatha generate variable lymphocyte receptors based on gene conversion. In higher vertebrates, T lymphocytes are equipped with highly diverse T cell receptors (TCRs) that are generated by gene assembly and VDJ recombination and are able to recognize self and non-self antigens. As these receptors are generated during life by somatic recombination they may not be subject to Darwinian selection, which leads to the risk of generating receptors that recognize self antigens. Therefore, vertebrates had to develop a system of quality control to ensure that autoreactive T cells are deleted during their development. Interestingly, thymus appearance coincides in the evolution with the generation of VDJ recombination. It is known that mice lacking the Foxn1 gene ("nude mice") do not have a thymus. When a revertable allele of Foxn1 was introduced in the genetic background of a nude mouse, a small thymus-like structure developed from single cells resulting in the selection of a normal diverse T cell repertoire. Thus, a single progenitor cell type, so called thymic epithelial stem cells can give rise to progenitors of the mTECs and cTECs. The Foxn1 gene can be traced back to the stage of transition of invertebrates to vertebrates about 500 Mio years ago and is a paradigmatic example for a situation in which birth of a single new gene can give rise to a new organ.

Vaccination against cancer (prophylactic and therapeutic)

H. zur Hausen (Heidelberg, Germany) gave a comprehensive overview on the incidence of microbial infection in cancer development and the effort undertaken to develop vaccines. The mechanisms involved in virus-linked tumorigenesis are best known, i.e., infection with DNA- or RNA-viruses such as the papilloma- (e.g., HPV in anogenital carcinomas), the herpes- (e.g., EBV in B-cell lymphomas), the hepadanae (e.g., HBV in hepatocellular carcinomas) or the flaviviridae (e.g., HCV in hepatocellular carcinomas) families. In the development of vaccines a breakthrough was obtained with the development of a prophylactic vaccine against HPV, which is able to prevent persistent infection with high-risk HPV types and as a consequence development of anogenital cancer. By using the L1 capsid protein of HPV type 6, 11, 16 and 18 as a target antigen (Gardasil®, Merck, which was recently released on the market) an antibody mediated immune response is elicited to neutralize these HPV types. A second prophylactic vaccine against HPV 16 and 18 L1 is expected to be approved soon (Cervarix®, GSK Biologicals).

T. Aebischer (Berlin, Germany) reported on vaccine development against *Helicobacter pylori* for controlling gastric disease and preventing cancer. It is currently believed that bacterial infection is promoting rather than igniting the carcinogenesis and that contact with the stem cells present in the gastric crypts is necessary for this process. HP infections are accompanied by an inflammatory reaction, with production of IL1-beta and TNF-alpha. At the same time, it blunts the immune effectors, inhibits phagocytosis and effector T cell activation while inducing $CD4^+CD25^+$ regulatory T cell (Treg) recruitment. Mice vaccinated with live *Salmonella* expressing the HP-derived urease antigen were shown to have a reduced bacterial load in the gastric epithelium and at optimal dosage could be protected against bacterial challenge. However, the field lacks adequate animal models to study the protection against HP-induced gastric cancer, since HP is a human-specific pathogen. Several trials have already addressed the safety and immunogenicity of *Salmonella* recombinant vectors in humans to seek proof of principle for a human vaccine. These studies provided evidence for immunity against HP infection in human beings which correlated with T cell activity. As the investigations revealed that strong antibody responses developing against the carrier were negatively correlated with T cell responses, *Salmonella*-based vaccines will need much improvement for efficient protection in humans.

In addition to the prophylactic vaccines, several colleagues also presented therapeutic vaccine approaches during the meeting. Y. Paterson (Philadelphia, USA) reports of

the development of a therapeutic vaccine against HPV. Her group proposes to use *Listeria monocytogenes* (Lm) as live and safe vaccine vectors. In contrast to other bacterial vectors (such as *Salmonella*), recombinant Listeria induced both $CD4^+$ and $CD8^+$ specific T cells. Moreover, the weak induction of neutralizing antibodies makes boosting with *Listeria* possible. In mice, Lm-LLO-HPV16 E7 (Lovaxin C®), in which E7 is secreted as fusion protein joined to a nonhemolytic listeriolysin O (LLO), induces strong $CD4^+$ and $CD8^+$ T cell responses with migratory capacity into HPV16 expressing TC-1 tumor, whereas a Lm-HPV16 E7 vaccine, not fused to LLO, induces Tregs, does not mature DCs nor facilitate T cell tumor infiltration. LLO perforates the phagolysosome so that the fusion protein can exit into the cytoplasm and enters the antigen processing pathway to be presented to cytotoxic T cells. Therapeutic vaccination with the Lm-LLO-E7 construct is able to eliminate tumors even in mice expressing the E7 as a transgene in the thyroid gland and in which peripheral tolerance may be expected. Similarly, vaccination with Lm-LLO-HER2/neu in HER2/neu mice developing autochthonous HER2/neu tumors delayed tumor growth. Tumor escape variants occasionally developed, in which the HER2/neu regions targeted by the Listeria-based vaccine were mutated. Thus, under immune pressure, immunoediting of tumor cells occurs through mutations of targeted tumor antigens. The conclusion from such experiments is that cancer vaccines should be able to prevent immune evasion by targeting tumor-indispensable epitopes and/or multiple antigens. Currently lovaxin C is being tested in cervical cancer patients.

In the development of therapeutic tumor vaccines against "self" tumor antigens, various approaches were undertaken, such as peptide-based vaccination. It is currently agreed that when minimal cytotoxic T lymphocyte (CTL) epitopes are used, a T-helper peptide, such as the universal $CD4^+$ T-help by PADRE, is in addition required for vaccine efficacy. Improvements can also be made by the addition of an adjuvant to the vaccine. However, P. Brossart (Tübingen, Germany) showed that the addition of low dose IL-2 as an adjuvant in a phase I/II trial with renal cell cancer (RCC) patients s.c. vaccinated with HLA-A2 restricted MUC-1-peptides (preloaded on DCs) and PADRE did not increase the persistence of $CD8^+$ T cell in vivo. In a total of 20 treated RCC patients, five experienced various degrees of tumor regression (one complete remission) and five others a stabilization of the progressive disease. In vitro tests showed the induction of a $CD8^+$ T cell response against the MUC-1-derived epitopes as well as against MUC-1+ tumor cell lines. Additionally, a specific T cell reactivity as measured by ELISPOT could be observed against epitopes not used for vaccination, suggesting that epitope spreading occurred upon vaccination in some patients, which may contribute to the clinical benefit of vaccination. Since the number of known target T cell epitopes for RCC is still limited, efforts are also being made to identify novel kidney-associated tumor antigens. The Adipophilin and the regulator of G protein signaling 5 (RGS5) are interesting candidates which are present in a limited number of normal tissues and overexpressed in the majority of human RCC. RGS5 is known to be expressed not only by tumor cells, but also by activated pericytes during tumor angiogenesis. Targeting and killing RGS5 expressing pericytes could potentially inhibit angiogenesis, thereby resulting in tumor growth control or delay. T cell epitopes derived from these two antigens that are restricted by HLA-A2 and -A3 have now been identified and could be used for immunotherapy.

Another approach for a therapeutic vaccine is to make use of DNA encoding the tumor specific antigen fused to a domain that induces $CD4^+$ T-helper cells. C. Ottensmeier (Southampton, UK) reported on the efficacy of such a naked DNA fusion vaccine design. This encodes the CTL epitope AH1 (which is a H2-L(d) restricted epitope derived from the endogeneous retroviral gene product gp70 expressed by colon carcinoma CT26) as tumor antigen coupled to domain 1 (pDOM) from fragment C of tetanus toxin (Fr-C, consisting of domain 1 and domain 2) known to only induce $CD4^+$ T cells. Since AH1, as most tumor antigens, is weakly immunogenic, deletion of dominant regions harboring both murine and human CTL epitopes in Fr-C (i.e., domain 2) directs the immune system towards the tumor antigen, thereby inducing strong $CD8^+$ anti-tumor immunity and long lasting memory in mice. On the base of the pre-clinical data, the group decided to initiate a first clinical trial in patients with prostate cancer, which is a slow growing tumor with prostate specific antigen (PSA) levels detectable in the serum as a measure for tumor progression or recurrence. The prostate specific membrane antigen (PSMA) is expressed 1,000-fold higher in prostate cancer than in normal tissue. The transmembrane domain of PSMA harbors an HLA-A2 restricted CTL epitope. Currently, the immunogenicity of this PSMA epitope fused with pDOM delivered i.m. is being studied in HLA-$A2^+$ prostate cancer patients. The study also assesses the effect of electroporation as a novel delivery strategy for DNA. The early data show for the first time in humans that the potency of the DNA fusion vaccine can be improved by delivery of the DNA with electroporation and can be further enhanced after sequential administration. This improvement included an increased humoral response against Fr-C and a faster CD4+ T cell response against pDOM. This route of administration was well tolerated by the patients. Also a significant increase in humoral response against pDOM was found, irrespective of pre-existing levels of antibody. The efficacy of this vaccination approach to induce CD8 responses and to control tumor progression is

to be determined with validated immunomonitoring analysis tools.

In the poster session, the use of peptide-based vaccination was also illustrated by M. Schmitt (Ulm, Germany). In a phase I/II trial, the immunological responses against the HLA class I peptide R3, derived from the receptor for hyaluronic acid mediated motility (RHAMM/CD168), was determined. RHAMM/CD168 is expressed on tumor cells of most patients with myeloid malignancies (AML, MDS, MM and CLL). The R3 peptide was emulsified in incomplete Freud's adjuvant (IFA) and given s.c. biweekly for a total of four times together with GM-CSF. The peptide vaccination was well tolerated and in half of the patients a significant increase of functional R3-specific effector $CD8^+$ T cells was found. In these responders, clinical efficacy was also reported with one complete remission, one partial remission and one haematological improvement. Because of these promising results, improvement of the vaccine targeting the RHAMM/CD168 as a tumor-specific antigen by exploiting other adjuvants is currently under investigation.

Another poster reported a phase I study by C. Gentilini (Berlin, Germany) for HLA-$A2^+$ high-risk AML patients after allogenic stem cell transplantation (HSCT). Biweekly vaccination with a WT-1 derived HLA-A2 epitope combined with keyhole limpet hemocyanin (KLH) and a daily dose of GM-CSF resulted in enhancement of the graft versus leukaemia-effect (GvL). In five of the six vaccinated patients, the immune responses were monitored in peripheral blood using tetramer analysis and IFNγ ELISPOT. Functional $CD8^+$ T cells were measured after vaccination, however, these responses disappeared 3 months after HSCT. In conclusion, WT-1 peptide vaccination could induce an effective anti-tumor response in patients with high-risk AML after HSCT. However, the possible enhancement of graft versus host-effect (GvH) due to the adjuvants warrants further clinical observations in a larger number of patients.

Another important aspect in the field of developing effective immune therapy is to identify new targets which clearly mark the tumor and not healthy tissues. A. Weinzierl (Tübingen, Germany) showed the so called "Tübingen approach" (A method that starts from tumor cells itselfs to identify, select and validate numbers of MHC/HLA class I-associated peptides derived from tumor-associated antigens) which can be used to identify potential target antigens not only by looking for mRNA expression but also by examining the MHC ligandome. E. Derhovanessian (Tübingen, Germany) described the direct elution of a novel HLA-B*1402-restricted epitope from the melanoma/melanocyte-specific protein, KU-MEL-1 from a melanoma cell line using affinity chromatography followed by mass spectrometric analysis. Additionally, another study by S. Jarmalavicius (Berlin, Germany) pointed out that protein modifications such as arginine methylation can be used to differentiate peptides expressed in normal versus melanoma tissue using mass spectrometry.

Inhibition of specific immunity

There is an increasing number of reports demonstrating that powerful mechanisms exist that can inhibit effective tumor-specific immunity; the key players as well as the underlying mechanisms of this inhibition are now being elucidated. H. von Boehmer (Boston, USA) is studying the conversion of peripheral naïve T cells to antigen-specific regulatory T-cells (Tregs): suboptimal delivery in the absence of costimulation (peptide infusion over a long period of time and at low concentrations or antigen delivery by minute amounts of DEC205-peptide fusion antibodies) results in Treg induction, which would be beneficial for the treatment of autoimmune diseases or in organ-transplanted patients. As a model, skin grafts derived from male mice are fully accepted in female mice which have been pretreated with adequate HY-peptide infusion. This correlates with a decrease in $CD4^+$ and $CD8^+$ anti-HY effector cells in vivo and an increase in $Foxp3^+$ T cells after skin graft transplantation. In mice, such long-lived Treg are $CD62L^+$ and recruited in antigen draining lymph node. Activated murine Treg are $CD103^+$ and can migrate to inflamed tissues. Foxp3 competes with AP-1(Jun/Fos) for its binding to nuclear factor of activated T cells (NFAT), a transcription factor regulating cytokine gene expression, in particular interleukin 2, in activated T cells. Treg function is dependent on the DNA binding NFAT-Foxp3 complex and hence Foxp3 does not induce relevant gene regulation in the absence of NFAT. In conditions involving suboptimal activation of T cells, Foxp3 is bound to NFAT. However, full activation through the TCR and via costimulatory molecules results in replacement of Foxp3 by AP-1. Whether lack of Treg conversion after costimulation depends on competition of AP-1 with Foxp3 for NFAT is presently unknown. Memory T cells, presumably because of high AP-1 content, cannot be converted into Treg by subimmunogenic antigenic stimulation even though they can be converted by retroviral Foxp3 transduction. In mice, subimmunogenic stimulation of T cells by tumor cells can result in tumor-specific Treg generation. Such $CD4^+$ Treg can inhibit tumor-specific $CD8^+$ T cells not only by altering their proliferation but also by decreasing their cytolytic activity, a mechanism which is dependent of TGF-β signaling in these $CD8^+$ T cells. Suppressed murine $CD8^+$ T cells do interact with their target cells, produce IFN-γ and are fully equipped with cytolytic molecules (granzyme B and perforin) but do not kill their targets. The suppressed $CD8^+$ T cells exhibit a delay in the exocytosis of their cytolytic

vesicles as evident by the delayed CD107a cell surface induction. Removal of Treg by antibody treatment can reverse the suppressed phenotype of $CD8^+$ T cells and result in tumor rejection. Such observations again emphasized the crucial role of Treg in impairing anti-cancer immunity at various stages of the immune response. Further knowledge of Treg cell development and functions is absolutely essential for the development of an effective therapeutic tumor vaccine.

J. Demengeot (Oeiras, Portugal) is studying the influence of microbial infection on the development of Tregs. Treg can obviously exert opposite effects here, decreasing inflammation and favoring the survival of infected animals on one hand, but increasing microbial load on the other. In fact, Tregs are equipped to respond to inflammatory signals and they express multiple surface molecules such as chemokine receptors, Toll-like receptors (TLRs 2, 4 and 6), CD25, the common gamma chain of the IL2/IL15 receptor and CD28. Thus, cross-talk can be established with effectors of the innate as well as adaptive immunity, through cell–cell contact or cytokines. In the NOD mouse model, where the animals spontaneously develop type I diabetes, the incidence of disease can be significantly decreased when small doses of LPS are administered weekly. This correlates with a significant increase in $CD4^+$ $Foxp3^+$ Tregs in the spleen. Moreover, protection is transmitted by transfer of the splenocytes of LPS-treated mice, but is abrogated if the splenocytes have been depleted from $CD25^+$ cells. In another model of autoimmune disease, RAG-1 deficient, TCR-transgenic (T/R-) mice that express exclusively myelin basic protein (MBP)-specific T cells, spontaneously develop experimental autoimmune encephalomyelitis (EAE), whereas RAG-1-competent transgenic animals (T/R+) remain healthy since they are protected by $CD4^+$ T cells (Treg) expressing endogenous TCRs. These T/R+ mice can be protected from EAE by immunization with the MBP-derived peptide that is recognized by the transgenic TCR when delivered in Complete Freund Adjuvant (CFA) and this is correlated here again with an expansion of $CD4^+$ $Foxp3^+$ T cells. Adoptive transfer of these $CD4^+$ $CD25^+$ TCR transgenic T cells from T/R+ donors to T/R-mice also prevented these mice from developing EAE. The observation that CFA and IFA, but not Alum, induces Treg development is intriguing and might have important and direct implications on the design of future anti-tumor vaccination approaches.

S.H. van der Burg (Leiden, The Netherlands) and his group investigates the role of regulatory T cells in HPV16-induced cervical cancer. In about 60% of healthy individuals, circulating T cells were found that are able to secrete IFN-γ and IL-5, to proliferate upon antigen-contact and to migrate to the epithelia of the skin following an intra-dermal challenge with peptides coded for HPV type 16 E2, E6 and E7. In contrast to most healthy persons, patients with pre-malignant high-grade cervical intraepithelial neoplasia (CIN) and cervical cancer patients fail to mount a specific immune response or displayed an impaired response against the early antigens of HPV16. Moreover, the lesions of these patients were strongly infiltrated by $CD4^+$ $Foxp3^+$ regulatory T cells. Interestingly, a proportion of these regulatory T cells were shown to be HPV-specific and able to suppress the function of naïve and effector $CD4^+$ T cells when stimulated with their cognate HPV-antigens. The impact of such a type of pre-existing HPV-specific immune responses were discussed in relation to vaccine strategies and pointed out that therapeutic vaccines may also boost unwanted immune responses.

Although T regs are the focus of much attention, it is also clear that other immune cell players are involved in inhibiting tumor specific immunity. V. Bronte (Padua, Italy) gave an overview on the relevance and function of myeloid derived suppressor cells ("MDSC") in mice and humans. Mouse MDSC co-express CD11b and Gr1 and can normally be found in the bone marrow of mice, whereas in the peripheral blood or the spleen they can only be detected in very low numbers. Nevertheless, their number in the periphery can increase in tumor-bearing mice and in states of chronic infection. Recent experiments have shown that the immune suppression found in mice bearing different carcinomas clearly depends on the presence of $Gr1^+$ $CD11b^+$ cells inside the tumors. In a model of colon cancer it could be found that the number of circulating $Gr1^+$ $CD11b^+$ cells significantly increases in the peripheral blood (up to 11.6%) in mice bearing CT26 tumors that were engineered to secrete GM-CSF. In contrast to $CD11b^+$ cells from naïve mice, the $CD11b^+$ cells from these tumor-bearing animals could completely suppress alloreactive $CD8^+$ T cells. MDSCs induce suppression of T cell function by two L-arginine modifying enzymes, the IFNγ inducible nitric oxide synthase 2 (NOS2) and the IL4/IL13 inducible arginase (ARG), respectively. The two enzymes can be activated independently, but only together do they engage a complex transcription program that results in suppression and apoptosis of T cells. In the tumor tissues from prostate carcinoma patients, ARG and NOS2 double-positive cancer cells can be found. When tumors were cultured for several days under conditions that allow maintenance of the tumor micro-environment, it was possible to detect fully equipped memory T cells that did not respond to activating stimuli such as PHA. A combination of ARG and NOS2-inhibitors could restore their function. As the systemic use of these inhibitors in patients is prohibited by their toxicity, the search for small molecular inhibitors has now begun and promises the development of a new category of drugs with anti-tumor effects. MDSCs form a heterogenic population of cells and this explains the need to identify specific

markers to distinguish different subtypes of MDSCs. In gene expression arrays of $CD11b^+$ cells from tumor-free and tumor-bearing mice, IL4Rα was found to be a potential marker in distinguishing MDSC sub-populations, as an $IL4R\alpha^+$ cell population up-regulates NOS2 and ARG and shows suppressive function in spleens of mice with different tumor types. First data from patients with RCC also suggests that $IL4R\alpha^+$ could represent a specific marker for tumor-induced MDSCs in humans. More and better markers for defining and monitoring MDSC sub-populations in patients will have to be identified.

Antibodies (effector mechanisms, new targets, therapy)

Until now the antibodies are the most successful immune therapeutics in cancer and several talks and posters focused on the effector-mechanisms involved, new methods to rapidly generate novel antibodies with favorable properties and the clinical use of new multivalent antibody-based constructs. J. Ravetch (New York, USA) demonstrated the therapeutic efficacy of IgG antibodies (such as anti-CD20 antibody in the treatment of lymphoma and adult T cell leukemia). IgG can bind (1) to neonatal FcR (FcRN), which increases the $t_{1/2}$ of IgG by protecting it from catabolism in the cell and recycle it to the cell surface back into circulation; (2) to complement (classical or alternative complement pathway) resulting in cell killing and (3) to Fc-gamma receptor (FcγR), thereby initiating ADCC by NK cells and inducing inflammation. This latter occurs when the FcγR is an activating receptor (type I or III). However, activation of the inhibitory FcγR (type II) can lead to peripheral tolerance and autoimmunity. The affinity of the IgG isotypes differs for the various FcRs; in mice the IgG2a has the highest affinity for activating FcγRs. Thus, the balance between the activating (A) and inhibitory (I) FcγR-mediated activity is important for therapeutic antibodies. To improve this balance, modifications were made at site Asn297 of the IgG. Fucosylation increased the A/I ratio resulting in an increase of antibody mediated cytotoxicity (i.e., ADCC) of the FcγR-expressing cells, cells whereas sialylation reduced this cytotoxic effect. Currently it is being investigated whether blocking of the FcγR-II, in addition to these modifications, might increase the efficacy of the IgG vaccine.

Additionally, the efficacy of Ab treatment can be explained by FcR polymorphisms. This is the case for CD20 Ab-based therapies which signals through the FcγRIII (CD16) receptor as reported by G. Weiner (Iowa City, USA). Modifications in the constant region of CD20 Ab enhance activation of NK cells and increase ADCC. In separate studies, fixation of complement, which is known to induce lysis of target cells in some conditions, can paradoxically block activation of NK cells. The concentration of mAb that activates NK cells is lower than the concentration that fixes complement. Thus, it may be possible to identify a dose of mAb that mediates ADCC but is low enough to prevent fixation of complement. In summary, for clinical applications, the polymorphism of the FcγR, the dose of Ab and the activation of complement proteins are important issues that need to be considered for improving current clinical benefit.

D. Neri (Zürich, Switzerland) identified Ab-targets from the vascular system first by biotinylation of vascular cell surface proteins and then by fishing out these proteins on a column followed by 2D peptide mapping. This is a high throughput system for identification of novel target antigens. Antibodies against these target antigens can then be raised in the delay of one week (Philochem innovating chemistry). When the Ab L19 was fused to bioactive molecules such as IL-2 or TNFα for targeting the vascularization specifically in tumor tissue, the therapeutic efficacy of the Ab in murine models was improved. Currently, a DNA encoded chemical library, in addition to the phage display library often used, is under development.

M. Sproll (Martinsried, Germany) presented another technology platform developed by Morphosys for high throughput selection and editing of human antibodies. It consists of a synthetic phage display library of more than 10^{10} Fab fragments (HuCAL®, GOLD) for rapid selection of binders against target antigens. An increase in Ab affinity (up to 3,000-fold) can then be obtained by diversifying the complementary determining regions (CDR) of the heavy and light chains against those from a CDR-library. Several products are being tested in animal models or in phase I/II clinical trials covering different research areas such as tumor therapy or Alzheimer disease.

Another form of antibody-construct for anti-tumor therapy was discussed by M. Penichet (Los Angeles, USA). Antibody fusion proteins are composed of the cytokines IL-2, IL-12, or GM-CSF genetically fused to a humanized IgG3 specific for the human HER2/*neu*. Overexpression of HER2/*neu* has been described in several malignancies such as breast and ovarian cancer and this overexpression is associated with poor prognosis. The main goal of this approach is to target sufficient quantities of the immunostimulatory cytokines to the site of HER2/*neu* expressing tumors to enhance the tumoricidal activity of the antibody and/or to elicit a tumor specific immune response. Human IgG3 was chosen because of its extended hinge region, which provides spacing and flexibility thereby facilitating simultaneous antigen and receptor binding. IgG3 is also very effective in ADCC and complement activation. These novel antibody fusion proteins [anti-HER2/*neu* IgG3-(human IL-2, also functional in mice), anti-HER2/*neu* IgG3-(murine IL-12), and anti-HER2/*neu* IgG3-(murine GM-CSF)], expressed in murine myeloma cells, are properly assembled, secreted, bind antigen and carry out cytokine

and antibody-related activities. Importantly, treatment of mice bearing human HER2/*neu* expressing tumors with these antibody fusion proteins, both as direct anti-tumor agents and as adjuvant of HER2/*neu* protein vaccination, results in significant anti-tumor activity under conditions in which the antibody alone fails to confer protection. Physical association between the antigen and the antibody fusion proteins is required to elicit the most effective protection, which involves humoral and cellular immune responses. In addition, long-term survivors are immune after challenge with non-HER2/*neu* expressing variants of the tumor cells, which might be due to epitope spreading. The low immunogenicity of the extracellular domain of HER2/*neu* (ECD^{HER2}) is due to the fact that although this molecule is readily internalized by dendritic cells, it is poorly processed due to its high level of glycosylation that results in early endosomal retention and recycling to the cell surface. However, anti-HER2/*neu* antibody-cytokine fusion proteins such as IgG3-(IL-2) redirect the trafficking of ECD^{HER2} to antigen processing compartments of dendritic cells resulting in effective antigen presentation.

Currently, tri-functional antibodies are being studied in clinical trials. As showed on two posters, both T. Leidig (Mainz, Germany) and B. Rodday (Mainz, Germany) used tri-functional antibodies (catumaxomab and ertumaxomab, Fresenius Biotech and TRION Pharma) to create the hypothetical tri-cell-complex consisting of a tumor cell (via EpCAM or HER-2/neu binding), T cell (via CD3 binding) and accessory cell (via CD16 or CD64 binding through FcR on NK, DC and macrophages) with the aim of inducing an effective anti-tumor response. Both groups analyzed PBMCs infiltrating the 3D tumor model called multicellular tumor spheroids and are focusing on the expression of cytokines and activation markers using ELISA or RT-PCR as readout system. Both studies concluded that catumaxomab caused a significant reduction of spheroid volume growth in a concentration-dependent fashion in vitro due to an increased incidence of tumor cell apoptosis caused by infiltrated leukocytes. Moreover, the leukocyte infiltration was dependent on the Ab concentration. These data support the effective usage of catumaxomab for immune therapy of patients with malignant ascites as recently shown in a phase II/III study. As malignant ascitis (containing the three above mentioned cell types to be targeted by the tri-functional antibody) is associated with poor prognosis it is very important to decrease the volume growth of the ascitis and maybe thereby be of clinical benefit for such patients (i.e., increased survival time).

Extra-session immunomonitoring

The Third Annual Meeting of the Participants of the CIMT Immunomonitoring Panel was held following the official end of the scientific program of CIMT 2007. This working group regroups 24 labs from nine different European countries and is dedicated to the standardization of assays applied for immunomonitoring. A satellite-session on immunomonitoring techniques was organized, with invited speakers presenting recent developments in the field. A. Thiel (Berlin, Germany) is an expert in detecting antigen-specific T cells by making use of the fast and transient up-regulation of CD154 (CD40L) on the surface of $CD4^+$ T cells upon antigen-contact. This assay, that has recently been published by this group, can be used for the detection of antigen-specific CD4+ T cells independent of cytokine production. Moreover, also combination with intracellular cytokine staining is possible. The simplest way of using the CD154-marker consists of a three color staining combining CD4 Ab and one defined cytokine of choice, but more complex experimental settings are feasible with combination of multiple colors conjugated to surface-markers and different cytokines allowing the simultaneous detection of complex populations of antigen-specific T cells of different phenotypes and functionalities.

H.M. Diepolder (Munich, Germany) showed that HLA class II-tetramers can be successfully used to systematically screen antigen-specific $CD4^+$ T cells in patients with Hepatitis C. As the MHC class II-tetramers are currently only available for a limited number of MHC class II alleles (mainly DR alleles) there is an urgent need for further development from companies that could make these reagents available and affordable for the scientific community. The combination of MHC class II-multimer staining with a subsequent enrichment step with anti-PE magnetic beads now also allows the detection of peripheral T cells present at frequencies which are below the currently established thresholds of detection. Once such a method has been extensively validated with dilution of antigen-specific T cell populations, it will be very useful for detecting tumor-specific $CD4^+$ T cells which can only be found in low numbers in patient material.

C.M. Britten (Leiden, The Netherlands) presented the results from two consecutive phases of an inter-laboratory testing project initiated by the CIMT monitoring panel. A total of 13 centres from six European countries participated in the study. The results of a first testing round revealed that the total number of cells analyzed was the most important determinant for the sensitive detection of antigen-specific $CD8^+$ T cells by tetramer staining. Analysis by ELISPOT was influenced by a combination of cell number and a resting phase after thawing of PBMCs. The experiments were repeated in a second phase but now the participants were asked to change their protocols according to the new guidelines distilled from the results of the first phase. The recommendations both improved the number of antigen-specific T cell responses that could be detected in participating labs

and decreased the variability between the laboratories. Such "two-step" inter-laboratory projects could define rational bases for immunomonitoring accepted international guidelines.

Conclusion

In this meeting we learned about the development of immunity in the thymus, a field that needs to be explored in more detail to understand and overcome tolerance against tumors. The development of both prophylactic and therapeutic vaccines against cancer was extensively discussed. Several approached were shown; i.e., peptides, viral or bacterial vectors for the antigen, DNA (fused to toxin) and antibodies. For determining the efficacy of anti-cancer vaccines sophisticated and complex immunomonitoring is required and should be standardized in order to compare results of clinical trials. Finally it was made clear that the inhibition of (vaccine-induced) immunity by for instance regulatory T cells or myeloid suppressor cells should be definitively considered in anti-tumor treatments. In short, it was again a very pleasant and stimulating meeting. The authors are looking forward to hear how the field continues to develop next year at CIMT 2008, which will take place in Mainz, 15−16 May.