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MEETING REPORT

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Abstract Cancer Immunotherapy 2005 was the third international meeting organized by the Association for Immunotherapy of Cancer (AIC). About 200 participants were attracted by the excellent scientific program that consisted of overview lectures from 25 international speakers in the plenary auditorium and four guided poster sessions during both days of the meeting. The first day of the symposium mainly focused on experience with, and new perspectives in, antibody therapy. On the second day of the meeting, organized as a joint conference together with the Combined Research Grant "Mechanisms of Tumor Defense and Therapeutic Intervention" funded by the German Research Council, the participants had the chance to gain deeper insights into the principles of antigen processing and the regulation of immune responses. Further topics that were discussed mainly in the poster sessions and in the special lecture given by M. Nishimura (Chicago, USA), were "cellular therapies" and "vaccination against cancer". The lectures selected for this report aim to provide an overview of the complete scientific program and give an impression of the lively atmosphere that could be felt from the first until the last session of CIMT 2005.

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The first day: antibody-based therapy

Thirty years after the description of continuous in vitro production of monoclonal antibodies (mAb) by Köhler and Milstein, considerable technological and financial efforts have been made to increase the efficiency of Ab in anticancer therapy. Eighteen different antibodies are now approved by the FDA for clinical usage, whereas about 400 reagents are being tested worldwide in diverse clinical trials.

Clinical trials

Current therapeutic approaches apply fully chimeric or humanized mAb either naked or complexed to toxins, cytokines, or radioactive components. Several clinical trials were presented, using Ab specific for CD20 (Rituximab, Ibritumomab tiuxetan), CD52 (Alemtuzumab), or CD33 (Gemtuzumab) for the treatment of T-, B-Cell lymphomas, or myeloid leukemias, with considerable success (J. Pagel, Seattle, USA; R. Repp, Kiel, Germany; M. Theobald, Mainz, Germany). The combination of anti-EGFR Ab (Cetuximab) with different regimes of chemotherapy or radiotherapy is being evaluated for colorectal carcinomas, head and neck cancer, and non-small cell lung cancers (N. Schleucher, Hamburg, Germany). For a variety of solid neoplasias, targeting mediators of angiogenesis represents an attractive alternative to classical tumor antigens. Thus, the VEGF pathway may be attacked using either Ab against VEGF itself (VEGF-A form) or its cellular receptor (VEGFR-2 is mainly implicated here). The humanized Ab Bevacizumab (anti-VEGF) is the first angiogenesis inhibitor currently approved for the treatment of cancer. A clear clinical benefit with acceptable side effects could be observed in combination with chemotherapy for colorectal carcinoma and more recently for renal cell cancer, lung cancer, and breast cancer (C. Emmanouilides, Los Angeles, USA). Another fast-growing technology is the

engineering of Ab-variable domains (Fv) coupled to toxins. Such Fv immunoconjugates are being tested with the aim of decreasing toxicity and increasing the effective dose delivered to the tumor. Thus, a disulfide-stabilized Fv portion from an anti-CD22 Ab fused to a fragment of Pseudomonas exotoxin A (BL22) was tested in phase I and II trials for patients with hairy cell leukemia (HCL), chronic lymphocytic leukemia, and non-Hodgkin's lymphoma (NHL) (I. Pastan, Bethesda, USA). This construct possesses a certain degree of toxicity, but when used at an appropriate dose, resulted in many complete responses in patients with drug-resistant HCL that are known to express a high level of the CD22 antigen.

Improving efficacy of antibodies

The recognized drawbacks of current reagents are immunogenicity (for mouse-derived Ab or chimeric human/mouse constructs) and toxicity (for immunoconjugates and radio-labeled), which limit treatment efficacy. To overcome the problem of immunogenicity, the generation of Ab in mice transgenic for human immunoglobulin genes is a promising strategy (J. van de Winkel, Utrecht, The Netherlands). With regard to toxic effects, J. Pagel (Seattle, USA) presented a pre-clinical model of "pre-targeting immunotherapy" in which the antigen-specific Ab (scFv) is linked to streptavidin and is first injected, followed by infusion of a cold-biotin clearing agent, and a final application of radiolabeled-biotin. One hour after application of the clearing agent, 90% of the free Ab was eliminated from the blood, so that up to five times higher dosage of radioactivity could be applied in this setting. Phase I trials now address the questions of safety, dosimetry, and efficacy of such approaches.

Not all antibodies targeting one defined tumor antigen are equal in their killing efficiency, and there is increasing need for a better understanding of the molecular basis for this apparently trivial observation. The primary mechanisms involved in the elimination of Ab-targeted cells are antibody-dependent cell cytotoxicity (ADCC), induction of apoptosis, and complement-dependent lysis (CDC). Different Ab binding to the same surface antigen have different properties in this respect, as illustrated by J. van de Winkel in his study of anti-CD20 Ab. Rituximab was shown to sustain consistent ADCC and CDC, but a novel anti-CD20 Ab (HuMax-CD20), recognizing a different epitope, was superior in inducing CDC as its main effector mechanism. Target cell-killing in vitro and in a SCID-mouse model in vivo was highly efficient, and the off-rate was slower for this newly developed Ab. This Ab was applied in a phase I/II trial in 40 NHL patients, and it revealed about 1/3 clinical responses in the 11 evaluable patients. In addition to the selection of Ab that induce good effector mechanisms, improved efficacy can be reached by other approaches, such as by increasing binding affinity by directed mutagenesis (anti-CD22 Ab for CLL, Ira Pastan), or modifying glycosylation, for example by co-expression of GnTIII, leading to the addition of bisecting *N*-acetylglucosamine, and decreased core fucosylation (J. Stieglmaier, Erlangen, Germany). The role of Fc-polymorphism as exemplified for FcγRIIIa (158 Val-Phe) should be investigated in greater detail, as it can obviously influence the patient's response to Ab therapy.

Finally, the importance of developing Ab that reacts specifically to the membrane-bound form of a given antigen—but not to the soluble form—and which can be released in vivo from tumor cells by physiological shedding was emphasized. This last point was discussed by I. Pastan for anti-CD30 Ab and J. Wijdenes (Besancon, France) for anti-CD25 and anti-CD138 Ab.

Defining new targets

U. Sahin (Mainz, Germany) presented an in silico strategy to identify new tumor-associated target antigens. Screening of 12,000 gene sequences from public databases finally revealed 25 candidates of the cancer germline antigen family, as verified by RT-PCR on RNA obtained from different tissues. These genes are under further analysis. In parallel, by targeting cancer-specific sequence alterations (splice variants) using multiple primer sets in RT-PCR, two promising candidates GT198 and TPTE were identified that are expressed in 90% of colon cancers and 40% of epithelial cancers, respectively.

Not only antigens from tumor tissue can be usefully targeted by antibodies, but also other molecules expressed by immune cells. For example, specific modulation of $CD4^+$ $CD25^+$ regulatory T cells (T reg) would be desirable in diverse clinical situations. One particular anti-CD4 Ab was found to activate selectively T regs in vitro (but not other $CD4^+$ effectors), whereas an anti-GITR Ab preferentially inhibits their function (J. Wijdenes). Such Ab might also find their way into cancer immunotherapy.

Interlaboratory testing project for T cell responses

One new activity of the Association for Immunotherapy of Cancer was also brought to the attention of the audience. A working group mainly focusing on the monitoring of immune responses to immunotherapy was founded at the beginning of this year. Members of this group aim at sharing their experience in different techniques and want to address problems in the field. As a consensus of the first meeting, it could be stated that until now, no correlation between clinical benefit and the detection of antigen-specific T-cell responses could be achieved. Since the induction of such T-cell responses upon vaccination is still seen as the most relevant effector mechanism of immunotherapy, the need for

standardized immune monitoring assays is high. The workgroup was therefore proposing to initiate an interlaboratory testing (called the ''CIMT Monitoring Panel''). A call for participants in the panel was presented shortly before the keynote lecture of the first day.

Keynote lecture: antigen recognition by TCR gene-modified cells

Transfer of selected TCR to human lymphocytes for generating high numbers of tumor-reactive effector cells could be a useful technology for adoptive immunotherapy of cancer. Several parameters should be considered when choosing a TCR for gene transfer, such as TCR avidity or CD8-dependency. Exploring this area, M. Nishimura studied the properties of and requirements for TCR-antigen recognition. First, the correlation between HLA-multimer binding and functionality is not absolute; this poor correlation may apply more often than commonly believed. Second, $CD4^+$ T cells bearing CD8-independent TCR can occasionally recognize their antigenic peptide (model T cells were TIL 1383I, specific for an HLA-A-restricted epitope from Tyrosinase) in the context of HLA-class I molecules on tumor cells and be fully functional. Retroviral transfer of their particular TCR into whole T lymphocyte populations could generate $CD4^+$ and $CD8^+$ T cells capable of recognizing tumor cells and able to elict various effector mechanisms. This duality may be exploited for the benefit of cancer patients. Another important finding was that when transferring the same TCR into different T cells, different functional properties, such as specific target-cell killing or IFN-γ production, could be observed. This suggests that intracellular components, for example signal transduction proteins, might be expressed at variable levels in T cells.

The second day

Antigen processing and presentation

The rational design of optimal vaccines requires in-depth knowledge of the fundamental principles of immunology. The first session of the second day addressed the molecular mechanisms involved in antigen processing and presentation. In his lecture, J. Yewdell (Bethesda, USA) emphasized the importance of the quantitative aspects in each step of antigen-processing and presentation, beginning from the synthesis of new proteins at the ribosome and ending with the presentation of adequately processed peptides via MHC class I molecules. Newly synthesized proteins can be divided into two subgroups with different life spans. Here, the majority of proteins are translated into stable products with a long half-life before final degradation. The second subgroup of translated proteins has a very short half-life before proteasomal degradation, and consists of ''short-lived'' or ''defective ribosomal products'' (SLiPs or DRiPs). Both fractions are mostly degraded to free amino acids and only a very small part leads to the generation of peptides that fit into and are presented via MHC class I molecules. Direct evidence was given that most antigenic peptides are derived from defective products of protein translation and not from the degradation of stable proteins (''DRiPs vs retirees''), as it was clearly shown that T-cell responses are linked to the rate of biosynthesis and not to the amount of antigen. Certain viral antigens are known to be very stable. Vesicular stomatitis virus nucleocapsid, for example, is not degraded until 12–24 h after viral infection. Nevertheless, T cells are able to specifically recognize infected target cells less than 45 min after infection. The fact that protein production is coupled to MHC class I presentation is the basis for the rapid T-cell response to viral antigens. As defective ribosomal products are the main source for peptides leading to efficient screening for viral infection, the term ''perfect use of imperfection'' is applicable.

Cross-presentation of antigens and licensing of T cells to kill is a main function of professional APCs. W. Heath (Melbourne, Australia) impressively showed that the enormous volume of research on DC subsets is not a purely academic task, but correlates with distinct functions in vivo. The number of new and different DC subsets that are described in the literature is steadily increasing, giving rise to a complicated and still inconsistent list. Nevertheless, more and more reproducible results support the subdivision into six ''major'' DC subsets, identified by the expression of different patterns of phenotypic markers. The first major subdivision is between plasmacytoid and conventional subsets. The main function of the plasmacytoid DC seems to be the production of IFNs that are important for blocking viral replication, rather than antigen presentation. The remaining five ''conventional'' DC subsets can be divided further into two categories: the three blood-derived DC subsets (termed blood-derived because they appear to become DC within the lymphoid tissues from precursors in the blood) and the two tissue-derived DCs. Blood-derived conventional DCs found in the spleen and lymphatic tissue can be separated based on their expression of CD4 and CD8α. One subset that expresses only CD8α (''CD8 DCs'') evolves as the predominant producer of IL-12 and was shown to play a central role in cross-priming and cross-tolerance. This DC-subset does not migrate but is resident in T cell areas of lymphatic tissue. Data were presented supporting the view that interstitial DC of the lung ($CD11b^-$, $CD8^-$, $CD205^+$) or dermal DC of the skin ($CD11b^+$ $CD8^-$, $CD205^+$) captures viral antigen in the periphery. Although lung interstitial DC could present viral antigens, this type of presentation was not detected for dermal DC. In both cases, however, evidence was provided that they pass on viral antigens to CD8 DCs via direct contact in the lymph nodes, providing a potential means to amplify immunity. It is still not clear what dictates cross-

priming vs cross-tolerance after antigen transfer to CD8α$^+$ DCs. To make things even more complicated, the sites of infection as well as the type of pathogen are further determinants of the quality and quantity of elicted effector immune responses.

Activation and suppression of immune responses

The concept that indoleamine 2,3-dioxygenase (IDO)-expressing APC can suppress T cell responses and induce tolerance or immune evasion is relatively new. Clear links between IDO activity, immunosuppression, and tolerance were described by A. Mellor (Augusta, USA), and the importance of further dissecting functional subpopulations of DC was emphasized. It is now clear that well-known phenotypic markers such as B220 or CD8α are no longer sufficient to precisely define a specific population of IDO-expressing DC. It was shown that DCs that express CD11c^{high}, B220, and CD19 on their surface, although representing only a small subset of the total pool of DC, are mainly responsible for IDO expression. The biological relevance of this subpopulation of IDO-competent DC is the ability to directly suppress the function of T cells. The molecular mechanisms by which IDO expression impairs T-cell function are now being elucidated in greater detail. There is some evidence that GCN-2 Kinase in T cells functions as a sensor and mediates arrest and anergy in response to IDO. The identification of IDO-expressing DC in tumor-draining LN provides a basis for future therapeutic approaches.

The induction of immune responses is not only dependent on the type and activation status of the APC, but also tightly controlled by regulatory T cells. E. Shevach (Bethesda, USA) proposed that there are two general categories of CD4$^+$ CD25$^+$ T reg cells. The first subset is a naturally occurring T reg population (nT reg) that develops during the normal process of T cell maturation in the thymus. The second subset of CD4$^+$ CD25$^+$ Treg (iT reg) can be induced after antigen exposure, as a consequence of low-affinity antigen or altered TCR signal transduction. Although distinct in nature, both T reg subsets can work in synchrony to control the outcome of adaptive immune responses. There is still much controversy in the definition of the different types of regulatory T cells, their origin, and their mechanism of action. More specific and stable markers and a nomenclature in respect to the complexity of T reg populations are therefore needed.

M. Bevan (Seattle, USA) presented data on the kinetics of the expansion, differentiation, and contraction of CD8$^+$ as well as CD4$^+$ T cells in response to antigenic stimulation. The quality and quantity of the signals needed to activate effector T cells and to maintain memory T cells differ for CD8$^+$ and CD4$^+$ T cell responses. In order to generate CD4$^+$ T cell memory, a longer presence of antigen is necessary. In *Listeria* infection, formation of CD8$^+$ T cell memory is independent of CD4$^+$ T cell help (as it occurs in class II deficient mice). Nevertheless CD4$^+$ T cell help is needed to maintain CD8$^+$ memory responses. Elucidating the mechanism by which CD4$^+$ T cells maintain CD8$^+$ memory will aid in designing more effective vaccines and explain the loss of immune function after depletion of CD4$^+$ T cells, as observed in HIV infection.

Further presentations demonstrated that the mechanisms underlying cross-presentation are only just beginning to be elucidated. Greater knowledge of the transit times and routes for soluble proteins after endocytosis and phagocytosis by APC, as described by H. Eisen (Cambridge, USA), will surely have an impact on future immunotherapies as well as on an understanding of linking innate with adaptive immunity by activation of extra and intracellular TLRs, as described by S. Bauer (Munich, Germany). Multi-photon intravital microscopy is a fascinating new method that now allows one to gain live and real-time insight into the kinetics of lymphocyte trafficking in vivo and their contact with endothelia and APC (Cornelia Halin, Zurich, Switzerland).

The poster sessions

The poster presentations held on May 12 and 13 were sub-divided into three parts (namely modulation of immune response, cellular therapy and vaccination, which roughly provided 50% of all posters). In all, 33 posters were the topics of lively discussions in guided poster tours by numerous participating researchers.

New methods to enhance the immunogenicity of vaccines were proposed by various groups. A. Sloots and colleagues (Frankfurt/Main, Germany) investigated whether specific targeting of antigens to APC in vivo can enhance the induction of ErbB2-specific T cell responses and increase antitumoral activity in the absence of any adjuvants or synthetic carriers. It was shown that intramuscular DNA vaccination with a vector containing a CTLA-4-ErbB2 fusion gene leads to the generation of ErbB2-specific antibodies and expansion of ErbB2-specific CD8$^+$ lymphocytes and subsequent protection of immunized mice. A new transcutaneous approach for delivery of antigenic peptides was developed in M. Radsak's group (Mainz, Germany). Here, T. Warger used a synthetic TLR7 ligand (Imiquimod provided as ointment) mixed with the antigenic peptide and achieved de novo priming against the OVA-derived SIINFEKL-epitope in C57/BL6 mice. Surprisingly, the group found nearly 100% lysis in an in vivo cytotoxicity assay after two transcutaneous immunizations. Additionally, they showed prolonged overall survival after two prophylactic transcutaneous immunizations in the EG7 tumor model. B. Scheel (Tübingen, Germany) presented data to demonstrate that besides coding for an antigen, in vitro transcribed mRNA has an additional adjuvant function. Using β-galactosidase-encoding RNActive (sequence stabilized mRNA) condensed with protamin,

she was able to show that stabilized ribonucleic acid incubated with various cell types leads to secretion of several cytokines in a TLR-dependent fashion.

Ongoing clinical vaccination studies were presented by two centers from Freiburg (Germany). B. Hildenbrand from the "Tumor Biology Center" reported a phase-I study using autologous DC loaded with PSA peptides after prestimulation with IFN-γ for HLA-A02$^+$ patients with hormone-refractory prostate cancer. So far, 15 patients were treated four times with DC. The vaccination was well tolerated and of 12 eligible patients, two showed a PSA decline, three a decrease in PSA-velocity, one no change and six an increase in PSA. H. Veelken from Freiburg University gave an update on the idiotype vaccination program for B-cell lymphoma, concluding that the phase I trial showed feasibility with a 90% success rate in providing the patient-specific recombinant Fab-fragment, which is given with adjuvant MF59 as an intradermal injection plus GM-CSF subcutaneously. In the ongoing phase II trial in low grade, untreated stage III/IV B-NHL, three of five follicular lymphoma patients achieved a PR after six vaccinations. Immune monitoring from one of these showed idiotype-specific humoral and cellular immune responses. Orphan drug status for the autologous idiotype vaccine has recently been granted by the European Medical Agency (EMEA). This shows that the enormous organizational and technical achievements in the production of patient-specific vaccine preparations are highly appreciated.

Several results were presented in attempts to discover new potential target candidates for future vaccination trials. A novel membrane protein NY-BR-1 that is overexpressed in breast cancer was introduced by I. Seil (Frankfurt/Main, Germany). M. Schmitt (Ulm, Germany) described the receptor of hyaluronic acid-mediated motility (RHAMM/CD168) as a promising target for vaccination in AML as this SEREX-identified antigen is expressed in 80% of AML but not in PBMC or CD34$^+$ stem cells. He identified two naturally processed HLA-A2-restricted epitopes and just recently initiated a phase I trial with subcutaneous peptide immunization. Using a reverse immunology approach, J. Mueller-Berghaus (Heidelberg, Germany) was able to identify two naturally processed HLA-A2-restricted epitopes from cTAGE-1, an antigen that is exclusively expressed in cutaneous T-cell lymphomas. The analysis of immune responses against the newly defined antigen candidates in patients is still ongoing. An advanced high-throughput approach to identify tumor-associated antigens and naturally processed peptides was presented by O. Schoor (Tübingen, Germany). This patient-specific approach combines gene-expression profiling of the patient's tumor with HLA ligand characterization using mass spectrometry. With this methodology, self peptides, known TAA, and novel TAA candidates were found.

Two approaches using bispecific antibodies were presented from G. Jung's group in Tübingen. L. Grosse-Hovest presented data on r28M, a bispecific single-chain antibody directed against the melanoma-associated-proteoglycan (MAPG) and CD28. After binding to tumor cells, the Ab induces "supra-agonistic" T-cell activation without an additional stimulus via the T-cell receptor, and leads to tumor cell lysis. *T. Otz* reported similar results with a bispecific single chain antibody targeting CD20. From T. Hermann, the successful lysis of glioblastoma cells using bispecific F(ab′)2 fragments with specificity for MAPG and the CD95 death receptor was reported. To utilize the ability of NK cells to enhance T-cell responses was the goal of a project presented by M. Jensen (Cologne, *Germany*). He showed that a bispecific antibody targeting CD3 and CD56 can not only redirect T cells toward CD56$^+$ tumors, but also act as an amplifier of antigen-specific T-cell responses by linking NK cells with T cells. The further development of these promising recombinant technologies and the evaluation of their clinical potential are just beginning.

A clinical study of cellular therapy with melanoma-reactive cell lines was recently initiated in Leiden (Netherlands). T. Ramwadhdoebé reported that a feasible protocol, based on the use of autologous and HLA-matched tumor cell lines as stimulators for PBMC, is now available. Sufficient numbers of GMP-grade tumor-reactive T cells could be generated in the majority of patients after 4–8 weeks of culture, and the first patient now has received the cells in a dose-escalation scheme. The presented results should encourage the extension of this approach. First experience in the use of a GMP-grade system for efficient CD8-depletion of donor lymphocyte infusions (DLI) were reported by R. Meyer (Mainz, Germany). After a reduced intensity in vivo T-cell depletion conditioning regimen, the patients received pre-emptive CD8-depleted DLI after withdrawal of immune suppression. Of 5 patients, only one developed severe GvHD. T cell response analysis revealed an increase in allo-directed as well as anti-viral immune reconstitution after CD8-depleted DLI. Additional studies of Langerhans-cell chimerism support the hypothesis of local allo-stimulation by persisting host-LC cells as contributing factors to GvHD and may provide the rationale for specific immune intervention to reduce this dangerous side effect of PBCST.

Conclusion

Cancer Immunotherapy 2005 covered a wide range of different topics and both the oral presentations and poster sessions contributed to the excellent scientific program of the meeting. It became clear that immunotherapy against cancer is a highly active field of fundamental, pre-clinical, and clinical research. The first day illustrated that the benefits of antibody immunotherapy are becoming available for more and more tumor entities and for an increasing number of patients. This development is based on the steady improvement of therapy regimens already approved as well as the continuous translation of new approaches and antibody constructs from the labs into clinical trials. Further

insights into the fundamental mechanisms of antigen processing and the regulation of immune responses, as demonstrated on the second day of the meeting, will lead to the rational design of new and optimized T cell-based specific therapies. The potential of immunotherapy has therefore not yet been fully exploited, and only the constellation of basic scientists and clinical researchers working hand in hand will lead to successful translation of the new concepts into effective treatments. The work of the relatively recently founded AIC is aiming to become more international in the future and we invite readers of this report to participate in its activities (see http://www.c-imt.org).