The manipulation of immunity

Conference on From Allergy to Cancer: New Perspectives for Therapeutic Vaccination

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The 89th Boehringer Ingelheim Fonds International Titisee Conference—'From Allergy to Cancer: New Perspectives for Therapeutic Vaccination'—took place between 17 and 21 March 2004 and was organized by R. Valenta and T. Brocker. The meeting brought together a diverse spectrum of scientists concerned with understanding the function of individual immune system components and exploiting this basic knowledge to manipulate the immune response.

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Wanted and unwanted immunity: thymic selection

The immune system has been evolutionarily selected such that it can execute wanted immune reactions and prevent unwanted immunity. There has been steady progress in understanding the essential role of the various mechanisms that result in immunity—or the lack thereof—and this knowledge is beginning to bear fruit as shown by the successful manipulation of immunity when natural processes fail.

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One key component of the immune system are the lymphocytes, each of which is equipped with a unique antigen-binding receptor that is carefully selected in order to match the needs of the individual organism. Developing T lymphocytes are screened for the specificity of their randomly generated receptors through processes termed positive and negative selection in the thymus (hence the name T lymphocytes; von Boehmer *et al*, 2003). This not only eliminates (by inducing apoptotic cell death) cells with receptors that are specific for self-antigens, but also positively selects (by avoiding premature death) cells that are able to detect pieces of foreign proteins when presented by the individual's own major histocompatibility (MHC)-encoded molecules. Class II MHC proteins present peptides from proteins that are endocytosed (such as bacterial proteins), whereas class I MHC molecules present peptides from proteins that are made inside the cell (such as viral proteins). There is also a process named cross-presentation through which proteins from a variety of different cells can be taken up and presented by class II or class I MHC molecules of antigen presenting cells such as dendritic cells (DCs). DCs endocytose proteins and, when activated, express costimulatory molecules that effectively stimulate T cells.

The positive selection process not only makes sure that the selected T cells are efficient in detecting these foreign peptides when bound by self-MHC molecules, but also aligns receptor specificity with functional potential. Therefore, CD4 T cells with receptors for class II MHC–peptide complexes help B cells to make antibodies to bacteria and bacterial toxins, whereas CD8 T cells with receptors for class I MHC–peptide complexes can develop cytolytic function that enables them to destroy virus-infected cells. It has also recently become clear that negative selection is an essential process for the survival of mammals, as a mutation in the gene that encodes the autoimmune regulatory protein (AIRE), which is involved in negative selection, leads to an early onset of autoimmunity that often affects oocytes and thus causes infertility (reviewed by von Boehmer *et al*, 2003).

The cell types in the thymus that are involved in positive and negative selection are still being disputed to some extent. T. Brocker (Munich, Germany) reported that class I or class II MHC antigens expressed under the control of tissue-specific promoters in either antigen-presenting DCs or B cells is insufficient for the positive

selection of CD4 and CD8 T cells. Conversely, expression on DCs is sufficient for negative selection of both CD4 and CD8 T cells inside the thymus. Expression of class II MHC molecules on B cells induces less efficient negative selection in the thymus, whereas class-I-expressing B cells fail to induce intrathymic negative selection. However, the latter can make CD8 T cells anergic—that is, resistant to antigenic stimulation—in peripheral (secondary) lymphoid organs where they migrate from the thymus. These studies of Brocker reinforce and extend earlier notions that under physiological conditions, thymic epithelial cells are most efficient in positive selection, whereas haematopoietic as well as epithelial cells are involved in negative selection.

The thymus is not only instrumental in the generation of CD4 helper and CD8 killer cells, but also the organ where so-called suppressor T cells, which express CD4 as well as CD25 on their cell surface and exhibit high levels of the Foxp3 transcription factor, appear for the first time in the developing organism. The selection of these cells is also essential for the prevention of unwanted autoimmunity as humans and mice deficient in the *Foxp3* gene suffer from severe early-onset autoimmunity. The generation of suppressor cells is dependent on ligands that bind with relatively high affinity to the T-cell receptor (TCR) either inside or outside the thymus as reported by H. von Boehmer (Boston, MA, USA; see below). Therefore, the regulation of wanted versus unwanted immunity begins with cellular selection and alignment of lymphocyte function with receptor specificity inside the thymus.

Unwanted immunity in secondary lymphoid organs

It is generally accepted that the selection mechanisms in primary lymphoid organs, even though essential, are insufficient to guarantee the absence of unwanted immune reactions. M. Nussenzweig (New York, NY, USA) discussed so-called 'recessive' mechanisms that prevent immunity outside the thymus: he reviewed published data showing that antigens can be targeted to DCs *in vivo* by using antibodies to DEC-205 (a DC surface protein) as carriers. This technique allows the effective delivery of antigens to class I and II MHC-I processing compartments, and the injection of nanograms of antigen is sufficient to induce tolerance to class I and II MHC antigens. Nussenzweig emphasized that steady state (mostly nonactivated) DCs targeted with antigen induce abortive T-cell responses that lead to tolerance and that DC activation with agents that induce maturation, such as CD40 ligation, lead to productive immunity. Two mechanisms of tolerance induction were discussed: T-cell deletion and the upregulation of CD5, which is a surface antigen that can raise TCR signalling thresholds. Nussenzweig showed that these tolerance effects are sufficiently profound to prevent experimental allergic encephalomyelitis in C57BL/6 mice injected with myelin oligodendrocyte glycoprotein peptide in Complete Freund's Adjuvant (CFA). He suggested that the physiological function of DCs is to maintain peripheral T-cell tolerance by continually presenting self-antigens (reviewed by Steinman *et al*, 2003).

Von Boehmer discussed the induction of tolerance in secondary lymphoid organs by the *de novo* induction of antigen-specific suppressor cells through continuous subcutaneous delivery of sub-immunogenic doses of peptides via osmotic pumps over a 14-day period. He showed that this process can take place in mice that lack a thymus and that the generated suppressor cells had a long lifespan in the absence of the inducing antigen. Moreover, after reactivation suppressor cells suppressed not only the effector function of T cells with the same antigenic specificity but also CD4 and CD8 T cells that

were in the vicinity of the suppressor cells. This was because they recognized antigens presented by the same antigen-presenting cells that reactivated the suppressor cells, a phenomenon known as 'bystander suppression'. Von Boehmer reported that this protocol could induce tolerance in the absence of any measurable immune response and could prevent diabetes in a transgenic murine disease model. The procedure therefore appears to represent an ideal approach for inducing antigen-specific unresponsiveness in the fully mature immune system even in older organisms in which the thymus is no longer functional. Von Boehmer expects this or similar procedures to eventually replace drugs such as cyclosporin, which are used to suppress unwanted immunity but have the disadvantage that they also suppress wanted immunity.

J. Ravetch (New York, NY, USA) discussed the impact of balancing activating and inhibitory signalling in immunity and tolerance. The focus of his presentation was on activating and inhibitory Fc receptors on B cells and phagocytes. FcRIII is an example of an activating receptor that triggers the enzymes phosphatidylinositol-3-OH kinase (PI(3)K), Syk and phospholipase C γ and their downstream mediators that are involved in the activation of B cells and macrophages. FcRII is an inhibitory receptor that functions by recruiting the SH2-domain-containing PI5 phosphatase to the cell membrane, thereby preventing the accumulation of the products of PI(3)K activation. Deleting the activating receptor prevents the development of systemic lupus erythematosus (SLE) in NZB mice through interference with the antibody-dependent effector phase of the response, but not with unwanted autoantibody production. By contrast, loss of the inhibitory receptor leads to an SLE-like disease in C57BL/6 female mice, which are not normally susceptible (Ravetch, 2004).

Clinical interference with unwanted immunity

One major topic of the meeting was the interference with, or the prevention of, unwanted immunity that results in the production of IgE antibodies that can bind to specific receptors on mast cells. This results in histamine release, which is involved in allergic responses. Another topic was the interference with transplant immunity that results in the rejection of important organ grafts such as kidney transplants.

G. Stingl (Vienna, Austria) discussed ongoing trials of omalizumab, which is an anti-IgE monoclonal antibody that could be used to treat atopic dermatitis. The antibody does not crosslink IgE on the surface of mast cells but does clear IgE from serum. He reported that in the ongoing trial, patients that responded with lower serum IgE levels also appeared to have lower levels of the Fc ϵ RI receptor for IgE on the surface of mast cells and DCs. It was proposed that the reduction of IgE and its receptor interferes with antigen presentation and immune amplification of the dermatitis reaction. Stingl suggested that mice fail to develop atopic dermatitis because they lack Fc ϵ RI expression. To test this idea and study the disease in more detail, he used a CD11c promoter to create a new murine model in which Fc ϵ RI α is expressed and forms a functional Fc ϵ RI receptor in CD11c⁺ DCs.

M.C. Larché (London, UK) presented his work on peptides derived from the major cat allergen, Fel d 1. He stressed that peptides do not induce the initial rapid phase of the allergic response because they fail to crosslink IgE on mast cells. Nevertheless, peptide administration in airways produced increased airway resistance and cellular infiltrates. Seven days after peptide inhalation there was an increase in CD4 T cells and eosinophilia in biopsy samples, but

this was less than that found with intact antigen delivered by the same route. There was no increase in cytokine levels in lavage fluids, histamines or leukotrienes. By contrast, patients given intradermal peptide became tolerant to subsequent challenge with the peptide. Tolerance correlated with the loss of T-cell proliferative responses *in vitro*, as well as increased interleukin 10 (IL-10) production, and Larché presented evidence that tolerance is mediated by active T-regulatory mechanisms. In the discussion, it was suggested that the different outcomes with inhaled and injected peptides might be due to the high activation state of the DCs in the lung of allergic individuals as opposed to resting DCs. The activated DCs in the lung might be expected to induce immunity whereas nonactivated DCs in the skin should induce T-cell tolerance.

R. Valenta (Vienna, Austria) reviewed the accomplishments in the area of allergen identification, gene cloning and structural analysis and focused on Bet v 1, the major birch pollen antigen that is a cause of allergy in many Europeans and North Americans. He described a strategy for desensitization based on vaccination with genetically modified allergen derivatives. He described two types of birch antigens that do not bind to the antibodies of birch-allergic patients and therefore do not induce immediate-type immune responses. These were birch antigen trimers and N- or C-terminal fragments of the Bet v 1 protein. Valenta showed the results of a double-blind, placebo-controlled trial in which these antigens were given before the allergy season, and patients were followed for one year. All of the modified antigens caused IgG responses. There was a positive correlation between clinical outcome and IgG production as well as decreased serum IgE levels and no seasonal increase in birch-reactive IgE production. The amount of Ig antibodies induced correlated with amelioration of disease, and Valenta discussed the possibility of being able to vaccinate against allergy using cloned and modified allergens in the future (Fig 1; Valenta *et al*, 2004).

M. Sykes (Boston, MA, USA) discussed the advantages of mixed bone marrow chimerism to prevent the rejection of allografts by inducing transplantation tolerance to allogeneic MHC antigens. Immune responses to such antigens can be strong and often, even in the presence of generally immunosuppressive drugs such as cyclosporin, can lead to the rejection of transplanted kidneys. A procedure of non-myeloablative conditioning by total body irradiation, and the injection of allogeneic bone marrow cells in combination with CD40 ligand antibodies that inhibit T-cell activation, was shown to result in mixed (syngeneic and allogeneic) bone marrow chimerism and to induce tolerance in CD4 and CD8 T cells. Even though alloreactive T cells were eventually deleted, tolerance was evident before the disappearance of such cells. No evidence for the generation of suppressor T cells was found. Patients with a mixture of allogeneic and syngeneic bone marrow cells develop blood cell chimerism that prevents the rejection of a kidney from the allogeneic bone marrow donor even when cyclosporin is withdrawn some time after transplantation. Interestingly, when mixed bone marrow chimerism is achieved in leukaemia patients, the transfer of mature T lymphocytes from the bone marrow donor can result in the eradication of the leukaemic cells without so-called graft versus host disease, which is more often observed in patients receiving allogeneic bone marrow after myeloablative pretreatment. Even though the precise mechanisms of preventing unwanted, while allowing wanted, immune responses in this system are not clear, the clinical success of this procedure makes it a worthwhile subject of more detailed studies in model systems (Sykes & Sachs, 2001).

Activating immune responses and immunological memory

Immune responses are initiated when TCRs bind to foreign peptides presented by self-MHC molecules on antigen-presenting cells, especially DCs. As described above, antigen presentation by non-activated DCs induces tolerance rather than immunity, and this is the reason why effective immunization has to be accompanied by 'adjuvants' that are required for DC activation. The immediate response of cells such as macrophages, certain B cells and DCs to bacterial products, usually contained in adjuvants, is called 'innate immunity' and is mediated by receptors such as the Toll-like receptors (TLRs) that resemble Toll trans-membrane proteins in *Drosophila*. These receptors are much less diverse than the antigen receptors of the adaptive immune system and their role in B- and T-cell immunity was discussed by several investigators.

A. Lanzavecchia (Bellinzona, Switzerland) discussed human B-cell memory. Immunological memory is defined by the heightened immune response to a previously encountered antigen. He reviewed experiments showing that purified human memory B cells are uniquely responsive to stimulation by TLR7 and TLR9, whereas naive B cells can upregulate TLRs and become TLR responsive only after antigen receptor stimulation. Naive B cells also differ from memory B cells in that T-cell help, CpG oligonucleotides (an adjuvant) and antigens are all required for *in vitro* activation, whereas memory B cells simply require CpG. Lanzavecchia showed a direct correlation between the number of memory B cells and serum antibody concentration and presented a model in which antibody responses in humans have three components: an initial rapid but short-lived burst of plasma cell production and antibody secretion; long-lived plasma cells with a half-life of 40 days; and persistent memory B-cell stimulation by TLRs and cytokines to maintain longer-lived antibody responses. These studies provide an explanation why antibodies against tetanus and measles can be detected for life in the apparent absence of the respective antigen.

H. Wagner (Munich, Germany) expanded on the theme that innate receptors are essential for T cell activation by discussing the biology of TLRs. TLRs are found either in endosomes (TLR3, 7, 8, 9) or on the cell surface (TLR2, 4, 5, 11 and CD14). Those that are in the endosomes are positioned to interact with nucleic acids released from viruses and bacteria that are partially digested in this compartment. The substrate specificity of TLR9 (double-stranded DNA), 7 and 8 (single-stranded RNA), and 3 (double-stranded RNA) are all consistent with this idea. TLRs function through distinct signalling pathways, and experiments with green fluorescent protein-labelled MyD88 showed endosomal recruitment of MyD88 on TLR activation by CpG. Wagner showed that TLR9 is in the endoplasmic reticulum in the resting state and is recruited to the endosomes on phagocytosis. CpG binding by TLR9 was pH-dependent in a manner consistent with its endosomal localization and binding was inhibited by chloroquine. Wagner suggested that the activity of chloroquine in SLE therapy might be related to the inhibition of B-cell activation by altering TLR activation thresholds in the lysosome.

After the efficient stimulation of T cells, which results in the generation of relatively short-lived effector cells, so-called 'memory' T cells can persist for long periods of time in the absence of antigen. These cells have the capacity to become effector cells much faster than naive T cells when re-encountering antigens. Like memory B cells, these cells can also be expanded by cytokines in the absence of the antigen that is recognized by their TCR. R. Zinkernagel (Zurich, Switzerland) referred to this type of memory

as 'academic memory' as, in his experience, such cells fail to protect from infection with microorganisms. He suggested that parameters of immunological memory, such as specialized memory cells, do not necessarily correlate with protection against re-infection. He proposed the alternative possibility that immunity is a low-level antigen-driven response that maintains T-cell activation and protective

antibody titres. In this model, protection by immunity disappears when antigen disappears (Zinkernagel, 2003). These two views of immunological memory responses—inherent special quality (academic memory) versus antigen-driven response—differ fundamentally. During the subsequent discussion, the question was raised as to why memory cells with special qualities have been selected in

Fig 1 | Structures of known allergens. All structures are available in the Protein Data Bank and the code for each is given. (1) Timothy grass pollen allergen Phl p 2 (crystal, 1.9 Å resolution, 1WHO). (2) Mite allergen Der p 2 (NMR structure, 1A9V). (3) Mite allergen Der f 2 (NMR structure, 1AHK). (4) Bovine lipocalin dander allergen Bos d 2 (crystal, 1.8 Å resolution, 1BJ7). (5) Horse allergen Equ c 1 (crystal, 2.3 Å resolution, 1EW3). (6) Mouse urinary protein Mus m 1 (crystal, 2.4 Å resolution, 1MUP). (7) Protein Llpr10.1A from yellow lupine (crystal, 1.95 Å resolution, 1ICX). (8) Birch pollen allergen Bet v 1 (crystal, 2.0 Å resolution 1BV1). (9) Bovine β -lactoglobulin (crystal, 1.8 Å resolution, 1BEB). (10) Cherry allergen Pru av 1 (NMR structure, 1E09). (11) Timothy grass pollen allergen Phl p 1 (crystal, 2.9 Å resolution, 1N10). (12) Human profilin (crystal, 2.0 Å resolution, 1FIL). (13) *Arabidopsis thaliana* profilin (crystal, 1.6 Å resolution, 3NUL). (14) Ragweed pollen allergen Amb T 5 (NMR structure, 1BBG). (15) Wasp venom allergen 5 (crystal, 1.9 Å resolution, 1QNX). (16) *Hevea latex* allergen Hev b 8 (crystal, 3.1 Å resolution, 1G5U). (17) Bee venom phospholipase A2 (crystal, 2.0 Å resolution, 1POC). (18) Birch pollen profilin (crystal, 2.4 Å resolution 1CQA). (19) Hen egg-white lysozyme (crystal, 1.8 Å resolution, 1BWH). (20) *Acanthamoeba castellanii* profilin (crystal, 2.3 Å resolution, 1F2K). (21) Yeast profilin (crystal, 2.3 Å resolution, 1YPR). (22) Bee venom hyaluronidase (crystal, 1.6 Å resolution, 1FCQ). (23) Rat urinary protein and its complex with a Hyaline Droplet Inducer (crystal, 2.9 Å resolution, 2A2G). (24) *Aspergillus fumigatus* manganese superoxide dismutase (crystal, 2.0 Å resolution, 1KKC). (25) Carp parvalbumin (crystal, 1.5 Å resolution, 4CPV). (26) Human serum albumin (crystal, 2.5 Å resolution, 1BJ5). (27) Functional domain of timothy grass pollen allergen Phl p 5b (crystal, 1.98 Å resolution 1L3P). (28) Timothy grass pollen allergen Phl p 6 (crystal, 2.8 Å resolution, 1NLX). (29) Tropomyosin (crystal, 7.0 Å resolution, 1C1G). (30) Timothy grass pollen allergen, Phl p 7 (crystal, 1.75 Å resolution, 1K9U). Figure kindly provided by R. Valenta.

evolution. It was pointed out that it is unlikely that they were selected for the purpose of academic entertainment as the term 'academic' memory might imply.

Vaccination

Vaccination refers to the effective application of antigen to generate protective immunity. As the first example of this phenomenon was the protection provided by cow (vacca) pox against human pox virus, vaccination is used as a synonym for such immunization.

T. Kündig (Zurich, Switzerland) reported that immunization with proteins, peptides, DNA or RNA was much more effective (up to 1,000-fold) when the agents were injected directly into lymph nodes or spleen when compared with the usual subcutaneous or intramuscular application. Conversely, the route of application was largely irrelevant when immunization was carried out with live microorganisms. Kündig also noted that much less CpG was required to boost immunity when injected directly into lymph nodes. This is important because higher doses of CpG have unwanted side effects. He also reported that injection of very small amounts of bee venom directly into the inguinal lymph node provided some protection against a subsequent bee-sting challenge. He suggested that subcutaneous or intramuscular application of allergen is very effective in preventing allergy, because only minute amounts reach the lymph node. Kündig also showed data on clinical trials in which the repeated application of tumour antigen directly into the inguinal lymph nodes resulted in effective activation such that up to 30% of the lymph node T cells showed specificity for the injected antigen.

Wagner presented a model for effective immunization that is based on CpG coupling to antigen, which activates antigen-presenting cells through TLRs after antigen uptake. He showed enhanced immunity to CpG-ovalbumin (OVA) conjugates at levels similar to bacteria-OVA. In the discussion following the presentation, Wagner suggested that antibody targeting to DCs might be combined with TLR activation by coupling CpGs to DEC-205 to deliver antigens and activation signals simultaneously for vaccination.

J. Iacomini (Boston, MA, USA) described a mouse model for the immunization of humans. He noted that humans, but not mice, are defective in the enzyme α -galactosyltransferase (α -GT) that is involved in the generation of α -Gal, which is present in a variety of tissues in mice. He produced knockout mice of α -GT, which, in contrast to normal mice, exhibited α -Gal antibodies in a subpopulation of B cells located in the marginal zone of B-cell follicles. Immunization of knockout mice but not normal mice with a protein (bovine serum albumin (BSA)) coupled to α -Gal resulted in excellent antibody responses to BSA even in the absence of adjuvants. Iacomini suggested that coupling antigens to α -Gal might become a strategy to vaccinate humans and that targeting marginal zone B cells so that they produce antibodies and present antigen might be an alternative adjuvant-free approach for effective immunization.

Passive immunization

The injection of preformed antibodies is referred to as passive immunization, and Lanzavecchia reported on the production of human monoclonal antibodies through the transformation of human B cells by Epstein-Barr virus. He suggested that such selected monoclonal antibodies might be protective against

serious infections and, in particular, he showed evidence of protection against the SARS corona virus.

Ravetch described experiments with rituximab in patients with lymphoma. These monoclonal antibodies are directed against the CD20 surface marker that is expressed on lymphoma as well as normal B cells. Successful treatment was dependent on the presence of activating FcR receptors, which presumably mediate the destruction of the lymphoma cells through macrophage antibody-mediated cytotoxicity. Therefore, the balance of activating and inactivating FcR in patients is likely to be crucial for the outcome of therapy using tumour-specific antibodies.

Immunity and cancer

Recent evidence suggests that the immune system can naturally provide some protection against cancer but even so, there have been many attempts to induce more effective immunity against tumours, albeit with limited success.

C. Melief (Leiden, The Netherlands) reported on a peptide-based vaccination approach that induces immunity against adenovirus-induced tumours. Antigen presentation on non-activated DCs resulted in proliferation but not accumulation of tumour-specific CD8 T cells owing to activation-induced cell death (reviewed by Steinman *et al*, 2003). When DCs were activated after the tumour had started to develop, vaccination with peptide had an anti-tumour effect in mouse model systems. He also described studies with melanoma cells and cytotoxic T lymphocytes (CTLs) that are specific for tyrosinase, which is involved in the production of melanin. Vaccination with granulocyte-macrophage colony-stimulating factor in combination with CTL-A4 receptor blockade resulted in tumour immunity as well as depigmentation of normal skin. The anti-tumour effect and depigmentation increased when suppressor cells were removed with anti-CD25 antibodies, and Melief presented evidence that suppressor cells prevent the expansion of CD8 T cells when secondary immunizations are performed.

H. Schreiber (Chicago, IL, USA) noted that the success of tumour therapy is inversely proportional to the duration of growth of solid tumours and is also dependent on the tumour stroma, which consists of nonmalignant activated fibroblasts, endothelial cells, extracellular matrix and cytokines. He inducibly expressed antigenic peptides in tumours, administered these tumours to mice and then analysed the response of CD8 T cells with transgenic receptors for the peptides. He compared tumours that express high and low levels of these antigens. The former tumours regressed in immunocompetent mice whereas the latter resulted in death. When tumours were given as a suspension they were always rejected. Rejection of solid tumours required cross-presentation of antigen by the tumour matrix, which somehow transports the antigen to local lymph nodes. An interesting experiment in which tumours expressing high and low levels of antigen were simultaneously injected yielded an unexpected result: even though both tumours are destroyed equally well by activated CD8 T cells, the high-expressing tumour was rejected whereas the low-expressing tumour generated antigenic loss variants that killed the animal. Schreiber concluded that for tumour rejection, the sessile stroma must also present the antigen so that it is destroyed by CD8 T cells. Therefore, in the low-expressing tumour, the antigen-expressing tumour cells are killed but the stroma is not, as there is too little cross-presented antigen. The intact stroma provides sufficient support for antigenic-loss variants to grow and kill the animal (Spirotto *et al*, 2004).

G. Schuler (Erlangen, Germany) and J. Gavilondo (La Habana, Cuba) reported on attempts to ameliorate tumour immunity in patients. Schuler immunized late-stage tumour-bearing patients with peptide-bearing DCs and monitored the immune response. He noted that peptide stimulation vanished after 48 hours and induced a poor γ -interferon and CD8 T-cell response. After repeated stimulation, CD8 T-cell responses increased and Schuler suggested that repeated antigenic stimulation in the presence of help for CD8 T-cell expansion and effector cell formation would lead to more effective immune responses. Gavilondo reported that increased DC activation and interference with angiogenesis, as well as with epidermal growth factor, could have beneficial effects in combating tumours.

Future directions

The panel discussion at the end of the meeting focused mostly on cancer, and it was emphasized that there is still a considerable lack of knowledge concerning tumour-specific antigens. The detection of such antigens might be enhanced by extracting RNA from tumours, expressing it in DCs and using these to induce specific T cells that define such antigens. It was also highlighted that compared with tumour vaccination, which is rarely successful, adoptive immunotherapy might be more effective. This would involve the transfer of expanded populations of tumour-specific CD8 T cells, possibly after receiving genes that encode tumour-specific TCRs into lymphopaenic patients in which the lymphopaenia provides a milieu for the uninhibited expansion and action of tumour-specific T cells.

Whereas effective tumour immunity still bears many question marks, it appears that the goals of interfering with unwanted immunity—such as allergy and certain forms of autoimmunity—might be achievable in the near future. The production of allergen variants that interfere with the action of natural allergens, as well as new technologies for inducing antigen-specific tolerance by recessive or dominant tolerance mechanisms, represent progress that justifies a more optimistic outlook for the future.

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REFERENCES

- Ravetch J (2003) in *Fundamental Immunology*, 5th edn (ed Paul, WE) 685–700. Philadelphia, USA: Lippincott-Raven
- Spiotto MT, Rowley DA, Schreiber H (2004) Bystander elimination of antigen loss variants in established tumours. *Nat Med* **10**: 294–298
- Steinman RM, Hawiger D, Nussenzweig M (2003) Tolerogenic dendritic cells. *Annu Rev Immunol* **21**: 685–711
- Sykes M, Sachs D (2001) Mixed chimerism. *Philos Trans R Soc Lond B Biol Sci* **356**: 707–726
- Valenta R, *et al* (2004) Immunotherapy of allergic disease. *Adv Immunol* **82**: 105–153
- von Boehmer H, Aifantis I, Gounari F, Azogui O, Haughn L, Apostolou I, Jaeckel E, Grassi F, Klein L (2003) Thymic selection revisited: how essential is it? *Immunol Rev* **191**: 62–78
- Zinkernagel RM (2003) On natural and artificial vaccinations. *Annu Rev Immunol* **21**: 515–546