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The 5th EFIS Tatra Immunology Conference on 'Molecular Determinants of T Cell Immunity' Held in the High Tatra Mountains, Slovakia, September 7–11, 2002☆

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In early September, over 100 scientists and students from Japan in the East, from San Diego in the west, and from pretty much everywhere in between, gathered at the Tatranské Zruby hotel and conference centre in northern Slovakia to focus mainly on immunoregulation (although some other aspects of T cell biology were also dealt with). This conference, held every two years, aspires to create an atmosphere akin to that of a Gordon conference. In that vein, scientists of all ranks and experience openly interacted over the course of a week, in the conference sessions, at meal times, or on afternoon trips to local sights. Some discussions were so far ranging that they even had to be continued in the small wooden cabin that constitutes the local bar! With an increasingly impressive faculty to lead the discussions, and with an ever-impressive set of trainees, there seems little doubt that the conference is very close to meeting its aspirations.

The meeting's focus acknowledged the unstoppable tide of reports over the past few years describing 'regulatory T cells' and various aspects of their biology. The important implication of this work is that the priming of a T cell does not necessarily provoke effector function. Rather, if priming conditions favor the activation of regulatory T cells, an animal may become tolerised. This could have serious consequences for immunisation strategies. Clearly, numerous questions are raised, most of which were considered at the conference. For example, just how many forms of T cell regulation exist? Are regulatory T cells always activated during an immune response in order to induce negative feedback regulation? Are regulatory T cells of a separate lineage, or does any mature T cell progenitor choose between becoming a regulatory T cell and an effector cell according to the conditions of priming and the consequent nature of the signals transduced from the T cell receptor (TCR) and associated co-stimulators? Are different outcomes provoked by dendritic cells (DC) of different types and/or at different stages of maturation? Does the form of antigen presentation vary under different circumstances? And what role do anatomy and microbial colonisation play in determining immunological outcomes?

To begin the conference, the multiple forms of immuno-regulation were summarised by *Jean-Francois Bach* (Paris), a true luminary who has made particularly important contributions to the study of autoimmunity. As a model of immune dysregulation, the Non-Obese Diabetic (NOD) mouse that ordinarily develops Type I diabetes, has much to offer. One can assess how immunoregulatory mechanisms affect the incidence and latency of the disease which usually takes a long time to develop (>12 weeks) following the commencement of overt pathologies such as insulitis at about 3 weeks of age.

(1) Th2 cells are obvious candidate suppressors of inflammatory diseases, and soluble antigen administration has been used to prime Th2 responses in NOD mice. Induced Th2 responses can protect against disease via by-stander suppression. This fails to occur in IL4-deficient NOD mice, and IL4 transgenic mice show reduced diabetes. Nonetheless, the prospect that this form of regulation is ordinarily of primary importance has been questioned by the fact that IL4-deficient NOD mice fail to show enhanced disease.

(2) $CD+^{4}CD25^{hi}$—regulatory T cells ('T-reg' cells) from pre-diabetic mice are protective when assessed in a

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co-transfer model in which diabetes is induced in recipients by $CD62L^+CD25^-$ T cells. The protection is blocked by neutralisation of TGFβ (which, controversially, Prof Bach claimed to be important for 'T-reg' cell maintenance) or of CTLA4. The 'T-reg' cells in NOD mice decline with age, whereas a short treatment with anti-CD3 as mice are becoming diabetic can cure disease, seemingly by favoring 'T-reg' cell activation and accumulation in the peri-pancreatic lymph nodes.

(3) NKT cells are present at overtly reduced frequency in NOD mice. Vαl4-Jα281 transgenic mice show slightly less diabetes, whereas it is accelerated in $CD1d^{-/-}$ mice. Since IL7 will rescue NKT cell development, the question was raised of whether NOD mice (and some human diabetics) suffer from IL7 dysfunction.

Cautionary notes considered in the question period included concern over the assessment of gene knockout mice on the NOD background, since it is essential that the effects of any particular gene mutation are judged against the full spectrum of NOD disease-association loci, of which there are very many. Thus, although IL4 deficient NOD mice fail to show enhanced disease, the same is true for IL10 deficient NOD mice, raising questions over whether the result with IL4 is confounded by variation in modifier genes that are segregating with the backcross.

Immunoregulation by DC was considered by *Muriel Moser* (Brussels) who reported on experiments using peptide pulsed DC subsets ($CD8\alpha\alpha^+$ or $CD8\alpha\alpha^-$) as adjuvants.

(1) Types of DC: $CD8\alpha\alpha^+$ DC express high levels of B7 and FasL, and are found primarily in conventional lymphoid sites, such as the T cell zones of the spleen; $CD8\alpha\alpha^-$ DC express lower levels of B7, and are additionally found in non-lymphoid tissues and the spleen marginal zone. When injected into the footpad, $CD8\alpha\alpha^+$ DC induced Th1 responses, whereas $CD8\alpha\alpha^-$ DC induced Th2 responses. DC from $lFN\gamma^{-/-}$ or $IL12^{-/-}$ mice show increased Th2 priming, whereas $CD8\alpha\alpha^-$ DC from $IL4^{-/-}$ mice still induce Th2 responses. However, Th2 priming by $CD8\alpha\alpha^-$ DC from $IL10^{-/-}$ mice does not induce Th2 responses.

(2) Maturation state of DC: immature ($CD24^{hi}$) DC drive naïve T cell proliferation but not effector function. The IL10R is somehow involved, because blocking it restores full T cell activation by immature DC. Immature DC also produce a lot of Type 1 interferon. The meeting considered whether or not this might be a constitutive mechanism of peripheral tolerance, with immature DC frequently presenting self-antigens in the absence of any danger.

Cautionary notes considered that the outcome of DC activity may vary dramatically according to conditions. Thus $CD8\alpha\alpha^+$ DC have also been reported to tolerise.

Immuno-regulation by microbes and the diet was considered by *Helena Tlaskalová* (Prague), who reminded the meeting that human beings are composed of 10^{13} eukaryotic cells and 10^{14} commensals. More than 80% of lymphoid cells are associated with mucosa, and an individual produces over 5 g of lumenal IgA per day! Importantly, microbes are co-determinants of immune responses. Thus, germ-free $IL2^{-/-}$, $TCR\alpha^{-/-}$, and $IL10^{-/-}$ mice fail to develop inflammatory bowel disease (IBD), (as do their conventionally housed counterparts), and the widely-used model of inducing IBD via transfer of $CD4^+CD25R\beta^{hi}$ cells to SCID mice does not work in germ-free animals. A mixed bacterial inoculum will restore disease, although no microbe has yet been identified that is solely responsible. Interestingly, T-reg cells from germ-free mice will protect SCIDs against transferred IBD, strongly suggesting that the T-reg cells do not require foreign antigen for selection and/or priming.

Of note: Some microbes may actually inhibit IBD, encouraging the prospect of a natural biotic treatment. Other diseases, Type I diabetes, and MRL-lpr lupus develop in germ-free mice, but instead appear to be affected by diet, a point considered at a molecular level in the last session of the meeting (*see below*).

Immuno-regulation induced in the thymus was presented by *Joost van Meerwijk* (Toulouse), who described imbalances in selection of the T cell repertoire when MHC expression was altered. For example, more T cells develop in mixed bone marrow chimaere of MHCI−/− II−/− into wild type recipients compared to wild type into wild type. This suggests that bone marrow derived MHC^+ cells ordinarily deplete the repertoire, consistent with an increased representation of self reactivity which was found among the additional T cells. Nevertheless, these cells do not induce graft versus host disease in syngeneic recipients, emphasising the 'split tolerance' concept of cells that are autoreactive in vitro but not in vivo. By contrast, an imbalance in thymic epithelial MHC expression (transgenic mice expressing MHC on cortical but not medullary cells) provokes the development of T cells that are autoreactive in vitro and in vivo. The conclusion that medullary epithelial cells ordinarily negatively select a substantial component of the repertoire is consistent with recent data that such cells express a broad panoply of tissue specific genes. The tolerance induced by medullary epithelial cells appears to be an anergic state that requires continual interaction with radio-resistant cells in the periphery, and that can be reversed. It does not require CD4 or CD25.

Of note: The important concept that some peripheral T cells may be positively selected on autoantigens and not negatively selected, and that such cells might play a regulatory role.

Tolerance via immunological ignorance was touched on by *Michal Novák* (Bratislava) who discussed prion expression. Splenic follicular dendritic cells (FDC) are primary reservoirs for the surface expression of prion diseases, and may act as a significant site of nucleation

of pathogenic complexes. A massive screening of 66 079 cattle sera for protease-resistant forms of Pr^{sc} revealed 11 positives. Analogous pathogenic protein-complex disease may form via aggregation of variant forms of the Tau protein. Although protein complexes may be nucleated in the heart of the immune system, protease resistance may severely inhibit antigen presentation, with the consequent likelihood that T cells remain ignorant of the antigens.

Tolerance via 'immuno-evasion' was an aspect of a presentation by *Heribert Stoiber* (Innsbruck) who reminded the meeting that HIV coats harbor proteins acquired from the host via budding and/or from the serum. Among the latter are complement proteins and complement inhibitors such as DAF and Factor H that will inhibit complement mediated virolysis. Hence the virus can escape innate immunological mechanisms. Antibodies against DAF and Factor H will render viruses more susceptible to virolysis. At the same time, the HIV coats can facilitate complement receptor mediated uptake by B cells, monocytes, and FDC particularly in the germinal centre. Such virus may be passed to T cells.

A session on *immunoregulation and disease* was led off by *Luciano Adorini* (Milan) who described tolerogenic DC expressing low levels of CD40, CD80, CD86, and MHC II, that can be induced by vitamin D3, widely used for immuno-suppression in psoriasis. The mechanism appears to involve multiple steps including but not limited to skewing of monocyte differentiation toward osteoclasts and away from DC maturation. The aggregate effect of vitamin D3 is to reduce IL12 production and to increase IL10. In a complex set of experiments vitamin D3 or its analogs was shown to improve tolerance of pancreatic transplant grafts, correlating with an increase of $CD4^+CD25^{hi}$ cells that will transfer suppression to secondary recipients. Moreover, vitamin D3 will suppress diabetes in NOD mice if administered early.

Scleroderma is a relentless, severely debilitating disease of immuno-regulation that was described by *Georg Wick* (Innsbruck), a founding organiser of the Tatra Conferences series. There is a chicken model, and moving back and forth between this and the human condition, Wick described evidence that apoptosis of endothelial cells may be a primary problem. This may be induced by antigen specific antibodies, present in afflicted individuals that target adult endothelial cells but not HUVECs. Serum transfers in the chicken provoked endothelial cell apoptosis. Additionally, scleroderma appears to be characterised by collagen dysregulation.

A focus on regulatory T cells was led off by *Ethan Shevach* (Bethesda) who reminded the meeting that $CD4^+CD25^+$ T regulatory cells (which he prefers to call suppressor T cells) play a physiologic role in immuno-regulation, as judged by the fact that their absence provokes autoimmune pathologies such as gastritis (as shown by S. Sakaguchi). The cells are substantially depleted from mice deficient in IL2, IL2Rα, IL2Rβ, Stat5A, CD40, CD40L, CD28, and B7.1/7.2. Additional hallmarks are their non-responsiveness to anti-CD3 and anti-CD28 or high concentrations of IL2. Following activation via the TCR, they suppress at 1 cell:4 effectors. But they cannot suppress truly strong responses, e.g. to high antigen or co-stimulator concentration or in the presence of large amounts of IL2. Indeed, a major effect appears to be the suppression of IL2 transcription in target T cells.

When cultured with anti-$CD3^+$IL2, T-reg cells can be expanded somewhat but they remain essentially anergic and acquire antigen non-specific suppressor activity.

Despite their involvement in vivo, IL10 and TGFβ are not necessary in vitro, where the mechanism of suppression requires cell–cell contact but is otherwise unelucidated. The use of microchip arrays identified TNFRI8 (GITR) expressed on resting T-reg cells but not on resting effector T cells. Antibodies to it ameliorate suppression, but its role is likely either a co-receptor in the suppression process or a survival receptor for resting T-reg cells akin to other TNFR members. It is not T-reg cell specific. The contribution of T-reg cells to disease was described in B6 mice that fully recover from Leishmania, but that do not fully clear the parasite. In the recovered state, 50% of T cells in the site local to the infection (the ear) are $CD25^{hi}$ and IL10 producers. The remaining cells are IFNγ producers, and their transfer to infected $RAG^{-/-}$ recipients will clear the parasite completely, whereas total cell transfers will not. Interestingly, when effector cells are permitted to clear the parasite, immunity was lost with it. Transfer of only T-reg cells worsens the disease in $RAG^{-/-}$ mice, strongly suggesting that the T-reg cells can target cells of the innate immune system, not just other T cells.

Of note: The lack of a T-reg-specific marker and the ignorance of the cells' mechanisms continue to prove major handicaps to this field. It was also pointed out in Discussion that $CD4^+$ $CD25^+$ T-reg cells are not the only cells that satisfy the criteria for physiologic suppression proposed by Shevach. For example, an inflammatory pathology spontaneously develops in mice lacking skin-associated gamma delta T cells, reconstitution of which rescues the normal phenotype. This form of immuno-regulation, like that imposed by T-reg cells, is under strict genetic control, with neither being particularly important on the C57.BL/6 background.

The potential to induce tolerance via use of T-reg cells was described by *Manuela Battaglia* (Milan). Rather than apply the Edmonton protocol of aggressive three-component immuno-suppressive drugs (sirolimus; tacrolimus; and anti-TAC) to graft recipients, Battaglia and colleagues substituted use of IL10. In diabetic mice

receiving allo-grafts, equivalent results in graft acceptance were obtained, but the use of IL10 seemed to achieve true tolerance rather than long-term chronic immuno-suppression.

The $CD8^+$ T-suppressor cell was described by *Nicole Sucia-Foca* (New York) based on studies of human transplant patients. In particular, $CD8^+CD28^-$ $CD27^+$ MHC-Class I-reactive cells were described to lack perforin or lytic activity and to reduce the increase in B7 expression ordinarily shown by DC during the early phases of T cell interaction. As judged by micro-array, the cells induce ILT3 and ILT4 on DC, that were claimed to induce anergy in responding $CD4^{-/-}$ T cells. The possibility that $CD4^+CD25^+$ T-reg cells may also induce ILT3/4 was considered, as was the prospect that such cells are themselves regulated by $CD8^+$ suppressor cells.

Of note: The Discussion session reviewed the checkered history of $CD8^+$ suppressor cells, and understandable concern was raised that immunologists do not follow the same ruinous path. It was agreed that vigilant attention needs to be paid to molecular mechanisms and to relevance in vivo of any proposed immuno-regulatory phenomena.

Andrey Antov (Cambridge, MA) presented more data on *$CD25^{hi}$-T-reg cell development*, claiming that IL2 was not required for the cells' development, but for their stable maintenance in the periphery. This is a controversial area. While the requirement for IL2 is unquestioned, others claim that the development of the cells is severely inhibited in the absence of IL2.

Arne Akbar (London) demonstrated that human $CD4^+CD25^+$ T cells are highly differentiated as defined by a short telomere and raised the possibility that these cells derive from antigen-specific T cells which have been anergized. He demonstrated that T-reg clones anergized by exposure to antigenic peptides in the absence of professional APC acquire T_R activity. However, using a new interesting in vivo model for experimental studying of $CD4^+CD25^+$ T-reg cells he could not find evidence that these cells were involved in the resolution phase of a delayed hypersensitivity response. Therefore, other inhibitory mechanisms may be involved. Alternatively, the retained hyperactive state of the antigen specific cells may mask the activity of any T-reg cell that might have been present.

Birgit Reipert (Vienna) addressed the problem of tolerance to therapeutic proteins and proposed the possibility of inducing T-reg cells using tolerogenic APC. She described experiments involving Factor VIII which is used in the treatment of haemophilia A and induces neutralizing antibody. In a mouse model of haemophilia she showed that blockade of CD40L does not induce tolerance, although it does inhibit the acute response to Factor VIII.

The meeting then considered *immuno-regulation at the level of antigen presentation, which, we were reminded, is a highly dynamic process*. *Harald Kropshofer* (Basel) described CDw78 as a determinant that is induced on B cells only alter activation and that reflects the re-organisation of MHC II glycoproteins into a membrane-associated 500 kD complex, distinct from a raft-associated unit, and including some unknown proteins, some tetraspanins CD81 and CD82, and HLA-DM which accelerates peptide loading. The MHC II molecules in this complex have bound to them a distinctive array of oligoclonal peptides, by comparison to that in rafts or in the membrane as a whole. Evidence suggests that the membrane complex increases the efficacy of T cell activation following engagement of MHC II by the TCR.

Immunoregulation at the level of epitope dominance has often been considered as a critical component of the dynamic between microbes and the immune system. If so, it seems paradoxical that there are so few established links between human (or mouse) MHC haplotype and susceptibility to infectious disease. Indeed, *Gabriele Niedermann* (Freiburg) presented data that there is essentially nothing that we don't see. Reminding us that $\sim$30% of synthesised proteins are immediately degraded, she considered the processing of the HIV Nef protein. Since Nef is the only HIV protein expressed in quiescent T cells prior to proviral integration, and because it down-regulates MHC I, it is a critical target for anti-HIV therapy. The N-terminus of MHC peptides is often generated by endoplasmic reticulum resident proteases, whereas the C-terminus is a direct product of the proteasome. There is an enormous repertoire of Nef epitopes, but they are clustered by proteasomal cleavages that favor hydrophobic amino acids and hydrophobic flanking residues. A very large proteasome complex, TPPII was identified that trims peptides downstream of the proteasome, as well as some de novo processing, to yield peptides with terminal lysine.

Marianne Boes (Boston) discussed evidence that *MHC II trafficking in cells is strongly polarised following T cell engagement*. Use of an MHC Class II-GFP fusion protein knock-in permitted MHC molecules to he visualised. Within minutes following T cell engagement, Langerhans cells (LC) displayed 'tubulation', the outgrowth of >20 μm long protrusions, compared to processes of only 1.5 μm in the absence of antigen. LPS makes a critical contribution, and high concentrations of LPS alone induce 'non-specific' tubulation, away from the directly targeted T cell. LC in $MyD88^{-/-}$ mice show fewer dendrites, and don't align properly in the epidermis. Moreover, the mice seem to show a defect in antigen presentation.

The requirements for and the nature of T cell activation were then considered, initially by *Lawrence Stern* (Boston) who uses powerful MHC oligomer reagents to demonstrate the apparent requirement for oligomers to activate T cells. Indications that naive $CD8^+$ T cells

from 2C TCR transgenic mice were activated by monomers were instead explained by T–T antigen presentation—thus, $CD8^+$ T cells from $MHCI^{-/-}$ mice were not activated in this way. Nevertheless, a contradictory conclusion was reached by *Alain Trautmann* (Paris), who imaged immunological synapses between T cells and DC. The meeting was reminded that such studies are rare, most synapses being visualised between T cells and either B cells or planar membrane bilayers. Whereas the latter clearly require antigen, DC synapses do not, although the resulting Ca^{2+} flux is weak and delayed. The synapses, which can be promoted even by $MHC^{-/-}$ mice, harbor CD45 and PKCθ, and high levels of phospho-tyrosine; as CD3 is recruited in, there is increased Ca^{2+} flux. The antigen-independent synapse seems to promote survival and low proliferation rates, with doubling times that were estimated by mathematical modelling to be $\sim$260h. CD2, LFA1, CD28, and ICAM1 may all play a role in this. When antigen is added in, data were presented that MHC-peptide monomers will stimulate antibody-immobilised adherent T cells. This provokes a true anti-apoptotic effect. PYK2 was implicated as central to the process of synapse formation; adherent cells have increased PYK2 levels, perhaps explaining that it, and not suspension T cells, can be activated by monomeric antigen. Tests in $PYK2^{-/-}$ mice remain to he completed.

Of note: The concept of antigen-independent signalling by DC might explain how DC arriving in the local lymph nodes can temporarily increase the survival of naïve T cells providing them with a longer time period to search for antigen during the afferent stage of an immune response.

The biological consequences of antigen receptor signalling at different stages of T cell maturation were described by *Federica Sallusto* (Bellinzona), who used responding T cell lines derived from single Th1 or Th2 categorised cells. Chromatin immunoprecipitation (ChIP) was used to show acquired acetylation at the IL4 and IFNγ promoters respectively, in polarised effector T cells. It was maintained for >20 generations; activated but non-polarised T cells did not show this, suggesting that the combination of anti-CD3 and anti-CD28 is insufficient to provoke the 'imprinting'. When candidate central memory cells derived from PBL were examined, they showed little acetylation, although they still responded very rapidly to secondary stimulation. Thus, it was proposed that cytokine memory is not imprinted at priming. In effector memory cells, imprinting was again evident, although more obviously in Th2 cells. As cells are maintained long term, most maintain the acetylation patterns, but it is not irreversible since some cells switch over. Indeed, there are many cytokine 'double producers', particularly among Th1 cells.

Of note: Polarisation but plasticity in the acquired immune response, leaving room for memory cells of a particular antigen specificity to he re-educated in terms of effector function, perhaps by a new set of contexts in which antigen is encountered secondarily.

The synapse was further examined by *Michael Dustin* (New York), who reminded us that despite its limitations, a planar membrane synapse including pMHC+ ICAM-1 will activate a TCR transgenic T cell. Within 30 s, LFA-ICAMI forms the focal point with the TCR on the concentric periphery. Over 5–60 min, the orientation reverses, forming the peripheral and central SMACs. In the nervous system, cadherins surround synaptic vesicle proteins. Mathematical models seem to mimic synapse formation well, and predict that weak interactions synergies strongly. As considered by Trautmann, synapses vary from case to case, and a hierarchy was proposed in which 'stop-signal' synapses, involving TCR, pMHC, CTLA4, and unknown DC ligands are in dynamic interplay with 'go-signals' that involve IL2, chemokines, and matrix signals. CD4 cells and CD8 cells may regulate synapses rather differently. In the absence of antigen, $CD8^+$ CTLs form adhesion rings in a continuously dynamic process. Only when antigen is added, the Golgi bodies move to a point aligned with the synapse. It was proposed that $CD8^+$ CTLs need only to release pre-formed granules, and that they therefore develop mature synapses very quickly. By contrast, the kinetics of naïve T cell synapses with splenic APC are much slower, and it may take hours before T cell proliferation is signalled, even though kinase activation was very transient.

The remaining speakers considered *molecular signals that ultimately set the thresholds for T cell responses*. *Amnon Altman* (San Diego) up-dated the meeting on PKCθ, an unusual PKC isoform involved in the cSMAC. $PKC\theta^{-/-}$ mice show defective T cell activation and maturation. Dominant negative $PKC\theta^{-/-}$ blocks downstream signalling of vav/rac, and appears to act earlier in the signalling hierarchy than conventional PKC (potentially upstream of TEK kinases) Indeed, PLCγ1 activation is impaired in $PKC\theta^{-/-}$ mice, as is recruitment of other PKCs to the membrane. Bc110 may be involved in raft recruitment of PKCθ.

The role of adaptor proteins was summarised by *Gary Koretzky* (Philadelphia), who focused on SLP76, an adaptor expressed in T cells, NK cells, macrophages, mast cells, and magakaryocytes, and a substrate of TCR tyrosine kinases. $SLP76^{-/-}$ mice have no peripheral T cells because of a block at the DN3, pre-TCR signalling stage of thymocyte development (there is no allelic exclusion). SLP76 is recruited to rafts via Gads and LAT that is constitutively raft-associated. Mixed bone marrow chimaerae in which $SLP76^{-/-}$ stem cells were transduced with altered SLP76 alleles were utilised to establish a structure-function relationship for SLP76.

Outside the T cell lineage, SLP76$^{-/-}$ fetuses have enlarged hearts and diffuse haemorrhages, which is attributed to an incomplete separation of the lymphatic ducts and blood vessels during development. It may not be cell autonomous since the phenotype is transferred by hematopoietic stem cells.

Takashi Saito (Chiba) described Gab2, an IRS-1 family adaptor, and also a ZAP70 substrate, but with extraordinary regulatory potential: following cytokine signalling, Gab2 is phosphorylated and acts as a signalling activator; following antigen receptor signalling, Gab2 is phosphorylated and acts as a signalling inhibitor. Again LAT is required for Gab2 phosphorylation, suggesting obligate raft association. There is a direct interaction with Gads that may compete with SLP76 for LAT-mediated raft association.

Thresholds can be set by negative regulation in the signalling webs as exemplified by csk, considered by *André Veillette* (Montreal). Csk inactivates Lck by phosphorylating Y505; it dynamically antagonizes CD45. Csk activity is enhanced as it interacts via its SH3 domain with phosphatases that simultaneously inactivate the positive regulators of signalling. Via its SH2 domain it interacts with PAG/Cbp that ensures localisation to lipid rafts. PAG is a transmembrane adaptor, and over-expression of it inhibits T cell activation, IL2 secretion and proliferation. Interestingly, IFNγ and IL4 production are not affected. PAG is normally extensively phosphorylated but it is quickly dephosphorylated following activation; this may be a critical aspect of T cell regulation. A minimal regulator is SAP which is simply an SH2 domain that interacts with SLAM and provokes fyn recruitment. An X-linked lymphoproliferative disease is attributable to mutations in SAP.

Immunoregulation by co-stimulation was described by *Oreste Acuto* (Paris), who clarified that although the role of CD28 is largely subsumed by high TCR occupancy, CD28-B7 substantially influences the capacity of the TCR to promote signalling. It also adds to CD2-based adhesion. On aggregate, the activities of CD28 seem to be mediated by Vav, upstream of P13-kinase.

Finally, and appropriate to the hearty food available at the conference, *Dietary regulation of immune signalling* was invoked by *Thomas Stulnig* (Vienna), with his evidence that polyunsaturated fatty acids (PUFAs) displace Lck, Fyn and LAT, but not PAG, from raft-like structures. PUFA inhibition affects JNK but neither Erk nor p38. NF-AT is also inhibited. Thus, IL2 responses and CD25 expression are altered. The mechanism seems to relate to an altered lipid composition of the inner and outer leaflets that is provoked by PUFA. The biological implications are apparent in the capacity to detect PUFA in lymph nodes.

The consideration paid to the molecular switches, both negative and positive, that undoubtedly regulate the T cell response to DC engagement (*with and even without antigen*) *was echoed in a very high quality poster session. Two posters were awarded prizes through the generosity of Nature Immunology and Nature Reviews Immunology. One poster* (*T. Brdicka et al.*) *described the very elegant molecular and biochemical identification of a new adaptor molecule, NTAL, about which the community will undoubtedly hear much more. The second prize rewarded excellent work by E. Briend et al, showing that a T cell may make an effector or regulatory response, contingent on the interaction of Notch and Notch ligands during T cell- APC engagement. As the participants gathered around a wonderful, and rather fiercesome camp fire for warm wine, barbecued food, and ethnic songs and dancing, deep questions remained, including the degree to which the regulatory phenotype is predetermined developmentally, or can be induced as part of a very plastic peripheral response. The quality of the young scientists attending encourages one to think that these issues will be addressed with superb experimental science that will avoid the serious pitfalls that confounded the analysis of suppressor T cells, 20 years ago. We look forward to learning more in 2004!*