

BASIC SCIENCE FOR THE CLINICIAN: T CELL REGULATION IN JUVENILE ARTHRITIS

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Abstract

The maintenance of immune tolerance is achieved through several mechanisms in the vertebrate immune system. For T cells these mechanisms include selection of T cells in the thymus with removal of potentially 'autoreactive' cells, as well as peripheral mechanisms including immune privilege sites, activation induced cell death and immunoregulatory cytokines which regulate or prevent responses to self. There is now much evidence for regulatory T cells (Treg) in the periphery: of these regulatory cells, the best characterised to date are the CD4⁺ CD25⁺ Treg cells. This paper reviews recent data on the various types of Treg and their proposed mechanisms of action, and summarises findings relating to Treg in juvenile idiopathic arthritis (JIA). Of interest is the recent demonstration that in the mild form of JIA known as persistent oligoarticular JIA, Treg are present and functional in the joint, and are at higher frequency than in the more severe, extended oligoarticular JIA. An understanding of how the balance between regulation and inflammation is controlled should allow us to design more specific and targeted therapies for the severe forms of arthritis in children, as well as other autoimmune diseases.

Introduction

Two central features of a healthy immune system are the ability to respond to a vast diversity of foreign microbes or pathogens, while at the same time preventing immune responses to self molecules. The first of these goals is achieved by antigen recognition by two inter-connected parts, the innate and the adaptive systems. The innate system recognises a set of relatively non variable molecules on microbes, using a limited number receptors known as Pattern Recognition Receptors (PRRs). In contrast, the adaptive system has evolved methods to generate vast arrays of highly variable receptors, expressed by B cells (as antibody) and T cells (T cell receptors). In the context of these variable receptors the immune system has co-evolved strategies to prevent potentially harmful responses to self proteins, known collectively as tolerance. In addition to being tolerant of self molecules, most healthy individuals maintain 'non-responsiveness' to a large number of dietary or inhaled antigens as well as commensal

gut bacteria. It is interesting that in many animal models in which the immune system has been disrupted, chronic inflammation of the bowel occurs (1).

For T lymphocytes, tolerance is critically dependent on the function of the thymus. Within this organ, high affinity self reactive T cells are removed (deleted) during development. A mechanism to facilitate this selection process has recently been elucidated. This involves the low level thymic expression of a wide range of self proteins from tissues all over the body specifically to 'educate' the developing T cells there, under control of the AIRE protein (2). However, central tolerance alone would be inadequate to ensure a safe level of non-reactivity. It is now clear that a set of mechanisms also exists in the peripheral immune system which is fundamental to maintaining immune tolerance and therefore to preventing autoimmune disease. One such mechanism which has recently come under intense investigation, is the contribution of T cells themselves, by so called 'regulatory' T cells (3, 4).

Mechanisms of control by regulatory T cells (Treg)

Regulatory T cells (Treg) were initially identified in mice by their ability to suppress proliferation of other cells in vitro, and to control autoimmune inflammation and disease in vivo (5-7). Removal of such cells leads to the spontaneous development of autoimmune pathology in mice, such as gastritis or colitis, though interestingly in some but not all models of animal arthritis (8, 9). A major type of regulatory T cells was identified by its surface expression of CD25, which is a component of the receptor for the cytokine IL-2 α (5). CD25⁺CD4⁺ T cells make up 5-10 % of normal CD4⁺ T cells in the blood of rodents and humans when defined by expression of CD25 of either medium or high levels (10, 11). Several studies have also suggested that the most functionally suppressive Treg reside predominantly within the population expressing very high levels of CD25 (CD25^{high}) (7). Despite their high expression of the IL-2R α (CD25), these cells do not divide readily in standard in vitro assays which are used to measure T cell proliferation (12). In vitro they require contact with their target cells in order to inhibit their proliferation (13, 14). However data suggesting a role for cytokines such as IL-10 or TGF β in the function of these Treg in vivo (15) as well as the demonstration that CD25⁺ Treg can in fact proliferate well in response to antigen in

vivo (16), suggest that the behaviour of Treg in vitro does not always reflect the in vivo situation. CD25⁺ Treg have been shown to exert inhibitory effects not only on other T cells, but also B cells and cells of the innate immune system including dendritic cells and NK cells (17).

The phenotype of CD25⁺ Treg is increasingly being characterised. However many of the proteins which are expressed on the surface of Treg, such as CTLA4 (cytotoxic T lymphocyte-associated protein-4), GITR (glucocorticoid-induced TNF receptor) and CD25 itself, are also increased upon activation of T cells, making it difficult to distinguish Treg from activated T cells, other than by their functional ability to suppress (18). The recent demonstration that the forkhead transcriptional regulator *foxp3* is highly expressed in CD25⁺ Treg, and that forced over-expression of *foxp3* induces a suppressive phenotype, has provided a specific tool with which to identify these cells (19, 20). This development is an example of 'convergence' of studies in mice and humans: the *foxp3* mutant mouse, known as the 'scurfy' mouse, (the product of the *foxp3* gene was originally called *scurfin*), has a phenotype which includes multiple autoimmune conditions and lymphocyte proliferation, from which these mice die within weeks of birth (21). Human patients in whom *foxp3* is deficient have also been described; they present with a syndrome of multiple autoimmune and inflammatory symptoms known as immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance syndrome (IPEX). This syndrome has been shown to be due to mutations in the human *foxp3* gene, which is located on the X chromosome (22). Many (perhaps most) CD25⁺ Treg arise from the thymus. However it has been demonstrated that CD25⁻ cells, when stimulated in vitro, or when influenced by CD25⁺ cells (23) or regulatory cytokines such as TGF β (24), may upregulate *foxp3* and acquire a regulatory phenotype. This raises the intriguing possibility that perhaps all T cells may be regulatory under certain conditions. It is now clear that Treg have an important role both in preventing autoimmune disease and also in the normal kinetics of immune responses to a wide range of pathogens (reviewed in 18). Therefore it seems likely that the generation of a set of regulatory T cells is part of the normal immune response, without which responses might continue, unchecked.

The mechanisms by which CD25⁺ Treg exert inhibition are still unclear, although one outcome of suppression is the blocking of transcription of IL-2, inhibiting production of this autocrine growth factor (25). Although in vitro the actions of Treg are dependent upon contact with their targets, and are not transferred by soluble factors, the surface expression of cytokines by Treg, in particular of TGF β , appears to play a role in suppression (26). Another interaction implicated in suppression by CD25⁺ Treg is that involving CTLA4 and its receptors CD80 and CD86 (27). The CD40/CD40L (CD154) interaction is also implicated in control of Treg function, since removal or blockade of this pathway during antigen exposure leads to increased CD25⁺ Treg-mediated suppression (28, 29).

Like all T cells, regulatory T cells require antigen presentation by specialised antigen presenting cells (APC) for function. Of the APC, the most potent are those known as dendritic cells (DC) and many studies indicate that DC are involved in generation of Treg (30). A bewildering diversity of in vitro systems have been used to generate such 'tolerogenic' DC, including culture in antigen in the absence of 'danger' signals (31), in the presence of steroids and Vitamin D (32) or IL-10 (33). Overall these studies suggest that either 'immature' or steady state DC are more likely to induce Treg and thereby tolerance, than fully activated DC: whether these represent separate developmental stages or pathways of differentiation is still unclear. However the concept of the regulatory DC as a potential tool to induce tolerance or even re-instate it during autoimmune pathology, is an emerging one (34).

In addition to CD4⁺CD25⁺ Treg, other types of regulatory T cell have been identified, in particular cells which are contact-independent but use cytokines such as IL-10 and TGF β . These cells, such as Tr1 cells (typically IL-10 producing) and Th3 cells (typically TGF β secreting) may develop in response to the effects of CD25⁺ Treg or independently (4). Both of these cytokines (IL-10 and TGF β) have been implicated in protection from autoimmunity in animal models and there has been some success in model systems in treating autoimmune pathology, including arthritis, by their targeted delivery to the inflamed site (35, 36).

Whether Treg cells have a specific 'repertoire' of antigens to which they preferentially respond is unclear, although already a wide range of specificities, both

self and foreign proteins, have been shown to be recognised by Treg (37). Evidence does suggest that certain self antigens appear to be immunodominant and involved in a protective response against autoimmunity. An example of this is the family of proteins known as heat shock proteins (hsp), highly conserved chaperone proteins which are upregulated in situations of cellular stress, and which have motifs that are shared across all species, from bacteria to mammals. In the adjuvant arthritis (AA) rat model of arthritis, T cells specific to hsp have been shown to protect against disease and nasal administration of hsp peptides reduced the onset and severity of the arthritis (38, 39).

Immunoregulation in juvenile idiopathic arthritis

In the context of this increased understanding of immune regulation by T cells and DC, a number of studies of juvenile idiopathic arthritis (JIA), which are discussed below, suggest that such regulation may play a role in some subtypes of childhood arthritis. JIA is a group of diseases which affects 1 in 1000 children under 16. The JIA subtypes have different clinical features, courses and genetic associations. Children whose disease is known (using the ILAR classification and criteria (40), as oligoarticular JIA (previously called pauciarticular) have 4 or less joints involved at presentation and in the first 6 months of disease. Within this group, two divergent groups then emerge: children whose arthritis remains mild, responds well to simple treatments such as NSAID and local joint injection, and frequently enters prolonged remission (known as persistent oligoarticular JIA), and those children in whom arthritis extends to many joints, may be severe and destructive, and can be difficult to control (extended oligoarticular JIA) (41). The latter may show some overlap with the subtype known as rheumatoid factor (RF)-negative polyarticular JIA, also a severe clinical subtype. There are genetic and immunological data to suggest that the mild phenotype of persistent oligoarticular JIA is in part due to immune regulation which may involve regulatory T cells and cytokines. If this is the case, this subgroup, frequently thought of as the 'easy-to-manage' patients by practising Paediatric Rheumatologists, may be of great importance to our ability to understand how severe arthritis progresses. Thus, the mild group may hold the clues which we need to unravel, in order to treat the severe forms of JIA more effectively.

We and others have shown that within the inflamed and highly vascular synovium of children with oligoarticular and polyarticular JIA, there is a dense infiltrate of activated, Th1 skewed T cells which contain highly expanded oligoclonal populations (42-45). Despite this, children with persistent oligoarticular JIA may make detectable levels of IL-4 from synovial T cells (45, 46). Since IL-4 is typically associated with a Th2 response, this may alter the balance of cytokines in the joints compared to those children with extended oligoarticular disease. In addition, it is interesting that the expression of CCR4 (a member of the CC chemokine receptor family)(itself since demonstrated to be expressed on CD25+ Treg) was shown to identify that synovial T cell population in JIA which has an increased IL-4: IFN gamma ratio (47).

Within the synovial fluid T cell population of many children with JIA, a population which responds well to human hsp60, (defined by proliferation of synovial T cells to hsp60 protein in vitro), is frequently present (48) . Strikingly, children with the highest response to hsp60 are those with persistent oligoarticular JIA, and in a prospective study, a strong response by peripheral blood mononuclear cells to hsp60 was found to be associated with good clinical outcome and remission of disease (49). These hsp specific T cells in patients with persistent oligoarticular JIA have since been shown to express CD30 (a marker also identified on Treg) and to make IL-10 (50). It is therefore possible that this hsp60 specific response represents part of an immunoregulatory process in oligoarticular JIA.

In collaboration with the group of Dr. B. Prakken, we have recently studied the CD4+CD25+ T cells in the joints of children with persistent and extended oligoarticular JIA. We have shown that children with persistent oligoarticular JIA have significantly higher ($p < 0.01$) levels of CD25+CD4+ cells in the joint than those with extended oligoarticular JIA (51). In addition, we found that that these CD25+ T cells contain cells which are suppressive in vitro, are positive for CTLA4, GITR, and CCR4 (all markers whose high expression is associated with regulatory T cells), and that they express foxp3 (51). Interestingly, the CD25+ cells in the joint included increased numbers of both CD25^{high} and CD25^{int} cells, (the latter expressing an intermediate level of CD25), but when analysed by CD25^{high} alone the difference between the two groups of patients was less marked. In the same study it was shown that the expression of

foxp3 was also greatly increased in the synovial CD25^{int} cells and that these cells can also suppress very effectively. Thus, at least in the inflamed joint, cells with an intermediate CD25 expression may also be suppressive.

An unexpected feature of the synovial fluid T cells from both JIA and rheumatoid arthritis patients has been the fact that despite their activated phenotype, they proliferate very poorly in vitro and contain few cells in active cell cycle, when analysed fresh from the joint (52-54). We have shown that this hypo-responsiveness to mitogenic stimuli which signal through the T cell receptor may be explained by the presence of CD25⁺ T cells. Thus, after separation of synovial T cells into CD25⁻ or CD25⁺, those which are CD25⁻, when cultured alone, respond as well as or more powerfully than their peripheral blood counterparts to stimuli through the TCR. In contrast the CD25⁺ synovial T cells alone are profoundly anergic (51). On the addition of cytokines such as IL-2 or IL15, or a specific antigen to which memory cells are present in the synovial T cells, a large proliferative response may be observed and the 'hypo-responsive' state can be overcome. By using a specific memory antigen, the influenza peptide HA307-319 to stimulate synovial T cells in vitro, and then staining with an MHC class II tetramer reagent which labels only T cells that are specific for this peptide (55), we have shown that in such stimulation assays, a significant part of the dividing synovial T cells are antigen non specific, (as judged by failure to bind a specific HLA-peptide tetramer reagent, Figure 1), and presumably able to respond to cytokines generated within the culture.

Thus we suggest that the CD4⁺ CD25⁺ population of synovial T cells includes a population of regulatory T cells. These cells have all the phenotypic hallmarks of CD25⁺ Treg, they do express foxp3 and they can suppress proliferation at least in vitro. Given that these CD25⁺ foxp3⁺ cells are also at higher frequency in the joints of children with persistent oligoarticular JIA, it is possible these cells contribute to the 'self-regulation' of pathology seen in this form of childhood arthritis.

Interestingly, Treg have now been demonstrated in a range of human autoimmune and infectious pathologies. With specific reference to arthritis, CD25⁺ Treg have been found to be present at high frequency in the joints of patients with rheumatoid arthritis (RA) (56). These cells were able to suppress the proliferation of

CD25⁺ cells from both blood and joint. These cells were also stable in number both between different inflamed joints and over time. The phenotype of the CD25⁺ synovial cells in RA was very similar to that of the CD25⁺ cells in JIA. However, in this study no correlation was seen between Treg in the joint and clinical features of the patients.

In addition to the functional studies on T cells in JIA already discussed, the genetics of JIA suggest that children with particular subtypes of JIA have genetic factors which alter the ability to regulate immune pathology. Collection of DNA samples from large cohorts of children with JIA has allowed such genetic studies with sufficient power to subdivide data by JIA subtype. In one such study where allelic differences in the promoter region controlling expression of the IL-10 cytokine, progression to extended oligoarticular disease was associated with an IL-10 haplotype ('ATA') which was shown to correlate with low IL-10 production in LPS-stimulated whole blood cultures (57). Further genetic studies have shown that while some 'risk' genes for JIA are shared across all JIA subtypes, and perhaps also with multiple autoimmune disorders, other genetic associations are subtype specific (58). For example some HLA risk associations differ between the two groups of oligoarticular JIA, in particular the haplotype DRB1*13-DQA1*01-DQB1*06 which is increased specifically in persistent oligoarticular JIA (33).

Harnessing regulatory mechanisms

There has been much progress recently in our understanding of how the immune system regulates itself, although several issues, such as the relationship between different types of Treg, their generation and their control, remain unresolved. The challenge now is to unravel the 'break points' at which tolerance is lost during autoimmune disease. Current evidence suggests that in one group of children with persistent oligoarticular arthritis the immune system may show effective regulation of an autoimmune inflammatory process: by understanding how this is achieved we may be able to harness these mechanisms to treat more severe forms of arthritis, both in adults and children. For example, in the future it may be possible to predict which children will evolve to severe disease, based upon the balance of inflammation and regulation in the joint, and to do this early in the disease process. If the differences in

Treg between disease subtypes are present early in the inflammatory process and can be shown to be predictive of disease course, then one could envisage a diagnostic test which would be based, for example, upon flow cytometry of cells from the first joint aspirate : this would be rapid, cheap, and simple to perform. In addition, it may be possible also to use regulatory pathways, such as that governed by foxp3, to design specific drugs which will tip the balance back towards regulation in arthritis and in other autoimmune diseases (59).

References

1. Podolsky, DK. Inflammatory bowel disease. *N Engl J Med* 2002; 347 (6): 417-29.
2. Anderson, MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002; 298 (5597): 1395-401.
3. Shevach, EM. Regulatory T cells in autoimmunity. *Annu Rev Immunol* 2000; 18 423-49.
4. Jonuleit, H and Schmitt E. The regulatory T cell family: distinct subsets and their interrelations. *J Immunol* 2003; 171 (12): 6323-7.
5. Sakaguchi, S, Sakaguchi N, Asano M, Itoh M and Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; 155 (3): 1151-64.
6. Read, S, Mauze S, Asseman C, Bean A, Coffman R and Powrie F. CD38+ CD45RB (low) CD4+ T cells: a population of T cells with immune regulatory activities in vitro. *Eur J Immunol* 1998; 28 (11): 3435-47.
7. Powrie, F, Leach MW, Mauze S, Menon S, Caddle LB and Coffman RL. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity* 1994; 1 (7): 553-62.

8. Morgan, ME, Suttmuller RP, Witteveen HJ, van Duivenvoorde LM, Zanelli E, Melief CJ, et al. CD25+ cell depletion hastens the onset of severe disease in collagen-induced arthritis. *Arthritis Rheum* 2003; 48 (5): 1452-60.
9. Bardos, T, Czipri M, Vermes C, Finnegan A, Mikecz K and Zhang J. CD4+CD25+ immunoregulatory T cells may not be involved in controlling autoimmune arthritis. *Arthritis Res Ther* 2003; 5 (2): R106-13.
10. Baecher-Allan, C, Brown JA, Freeman GJ and Hafler DA. CD4+CD25high regulatory cells in human peripheral blood. *J Immunol* 2001; 167 (3): 1245-53.
11. Ng, WF, Duggan PJ, Ponchel F, Matarese G, Lombardi G, Edwards AD, et al. Human CD4(+)CD25(+) cells: a naturally occurring population of regulatory T cells. *Blood* 2001; 98 (9): 2736-44.
12. Taams, LS, Smith J, Rustin MH, Salmon M, Poulter LW and Akbar AN. Human anergic/suppressive CD4(+)CD25(+) T cells: a highly differentiated and apoptosis-prone population. *Eur J Immunol* 2001; 31 (4): 1122-31.
13. Jonuleit, H, Schmitt E, Stassen M, Tuettenberg A, Knop J and Enk AH. Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. *J Exp Med* 2001; 193 (11): 1285-94.
14. Dieckmann, D, Bruett CH, Ploettner H, Lutz MB and Schuler G. Human CD4(+)CD25(+) regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells. *J Exp Med* 2002; 196 (2): 247-53.
15. Asseman, C, Mauze S, Leach MW, Coffman RL and Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 1999; 190 (7): 995-1004.
16. Walker, LS, Chodos A, Eggena M, Dooms H and Abbas AK. Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. *J Exp Med* 2003; 198 (2): 249-58.
17. Maloy, KJ, Salaun L, Cahill R, Dougan G, Saunders NJ and Powrie F. CD4+CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. *J Exp Med* 2003; 197 (1): 111-9.

18. Gavin, M and Rudensky A. Control of immune homeostasis by naturally arising regulatory CD4+ T cells. *Curr Opin Immunol* 2003; 15 (6): 690-6.
19. Hori, S, Nomura T and Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; 299 (5609): 1057-61.
20. Fontenot, JD, Gavin MA and Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; 4 (4): 330-6.
21. Khattri, R, Cox T, Yasayko SA and Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat Immunol* 2003; 4 (4): 337-42.
22. Bennett, CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001; 27 (1): 20-1.
23. Jonuleit, H, Schmitt E, Kakirman H, Stassen M, Knop J and Enk AH. Infectious tolerance: human CD25(+) regulatory T cells convey suppressor activity to conventional CD4(+) T helper cells. *J Exp Med* 2002; 196 (2): 255-60.
24. Chen, W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF β induction of transcription factor Foxp3. *J Exp Med* 2003; 198 (12): 1875-86.
25. Thornton, AM and Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* 1998; 188 (2): 287-96.
26. Nakamura, K, Kitani A and Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J Exp Med* 2001; 194 (5): 629-44.
27. Takahashi, T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 2000; 192 (2): 303-10.

28. Jarvinen, LZ, Blazar BR, Adeyi OA, Strom TB and Noelle RJ. CD154 on the surface of CD4+CD25+ regulatory T cells contributes to skin transplant tolerance. *Transplantation* 2003; 76 (9): 1375-9.
29. Cron, RQ. CD154 transcriptional regulation in primary human CD4 T cells. *Immunol Res* 2003; 27 (2-3): 185-202.
30. Mahnke, K, Schmitt E, Bonifaz L, Enk AH and Jonuleit H. Immature, but not inactive: the tolerogenic function of immature dendritic cells. *Immunol Cell Biol* 2002; 80 (5): 477-83.
31. Finkelman, FD, Lees A, Birnbaum R, Gause WC and Morris SC. Dendritic cells can present antigen in vivo in a tolerogenic or immunogenic fashion. *J Immunol* 1996; 157 (4): 1406-14.
32. Barrat, FJ, Cua DJ, Boonstra A, Richards DF, Crain C, Savelkoul HF, et al. In vitro generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J Exp Med* 2002; 195 (5): 603-16.
33. Steinbrink, K, Wolfl M, Jonuleit H, Knop J and Enk AH. Induction of tolerance by IL-10-treated dendritic cells. *J Immunol* 1997; 159 (10): 4772-80.
34. Steinman, RM, Hawiger D and Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003; 21 685-711.
35. Chernajovsky, Y, Adams G, Triantaphyllopoulos K, Ledda MF and Podhajcer OL. Pathogenic lymphoid cells engineered to express TGF β 1 ameliorate disease in a collagen-induced arthritis model. *Gene Ther* 1997; 4 (6): 553-9.
36. Fellowes, R, Etheridge CJ, Coade S, Cooper RG, Stewart L, Miller AD, et al. Amelioration of established collagen induced arthritis by systemic IL-10 gene delivery. *Gene Ther* 2000; 7 (11): 967-77.
37. Sakaguchi, S, Hori S, Fukui Y, Sasazuki T, Sakaguchi N and Takahashi T. Thymic generation and selection of CD25+CD4+ regulatory T cells: implications of their broad repertoire and high self-reactivity for the maintenance of immunological self-tolerance. *Novartis Found Symp* 2003; 252 6-16; discussion 16-23, 106-14.

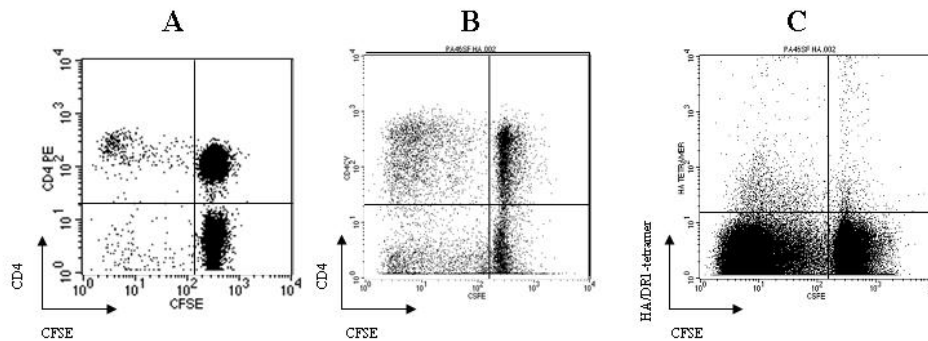
38. Prakken, BJ, Roord S, van Kooten PJ, Wagenaar JP, van Eden W, Albani S, et al. Inhibition of adjuvant-induced arthritis by interleukin-10-driven regulatory cells induced via nasal administration of a peptide analog of an arthritis-related heat-shock protein 60 T cell epitope. *Arthritis Rheum* 2002; 46 (7): 1937-46.
39. Prakken, BJ, Roord S, Ronaghy A, Wauben M, Albani S and van Eden W. Heat shock protein 60 and adjuvant arthritis: a model for T cell regulation in human arthritis. *Springer Semin Immunopathol* 2003; 25 (1): 47-63.
40. Petty, RE, Southwood TR, Baum J, Bhattay E, Glass DN, Manners P, et al. Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. *J Rheumatol* 1998; 25 (10): 1991-94.
41. Woo, P and Wedderburn LR. Juvenile chronic arthritis. *Lancet* 1998; 351 (9107): 969-73.
42. Thompson, SD, Murray KJ, Grom AA, Passo MH, Choi E and Glass DN. Comparative sequence analysis of the human T cell receptor beta chain in juvenile rheumatoid arthritis and juvenile spondylarthropathies: evidence for antigenic selection of T cells in the synovium. *Arthritis Rheum* 1998; 41 (3): 482-97.
43. Wedderburn, LR, Maini MK, Patel A, Beverley PCL and Woo P. Molecular fingerprinting reveals non-overlapping T cell oligoclonality between an inflamed site and peripheral blood. *Int Immunol* 1999; 11 (4): 535-43.
44. Wedderburn, LR, Patel A, Varsani H and Woo P. Divergence in the degree of clonal expansions in inflammatory T cell sub-populations mirrors HLA-associated risk alleles in genetically and clinically distinct subtypes of childhood arthritis. *Int Immunol* 2001; 13 (12): 1541 – 50.
45. Wedderburn, LR, Robinson N, Patel A, Varsani H and Woo P. Selective recruitment of polarized T cells expressing CCR5 and CXCR3 to the inflamed joints of children with juvenile idiopathic arthritis. *Arthritis Rheum* 2000; 43 (4): 765-74.
46. Murray, KJ, Grom AA, Thompson SD, Lieuwen D, Passo MH and Glass DN. Contrasting cytokine profiles in the synovium of different forms of juvenile

- rheumatoid arthritis and juvenile spondyloarthropathy: prominence of interleukin 4 in restricted disease. *J Rheumatol* 1998; 25 (7): 1388-98.
47. Thompson, SD, Luyrink LK, Graham TB, Tsoras M, Ryan M, Passo MH, et al. Chemokine receptor CCR4 on CD4+ T cells in juvenile rheumatoid arthritis synovial fluid defines a subset of cells with increased IL- 4:IFN-gamma mRNA ratios. *J Immunol* 2001; 166 (11): 6899-906.
 48. de Graeff-Meeder, ER, van Eden W, Rijkers GT, Prakken BJ, Kuis W, Voorhorst-Ogink MM, et al. Juvenile chronic arthritis: T cell reactivity to human HSP60 in patients with a favorable course of arthritis. *J Clin Invest* 1995; 95 (3): 934-40.
 49. Prakken, AB, van Eden W, Rijkers GT, Kuis W, Toebes EA, de Graeff-Meeder ER, et al. Autoreactivity to human heat-shock protein 60 predicts disease remission in oligoarticular juvenile rheumatoid arthritis. *Arthritis Rheum* 1996; 39 (11): 1826-32.
 50. de Kleer, IM, Kamphuis SM, Rijkers GT, Scholtens L, Gordon G, De Jager W, et al. The spontaneous remission of juvenile idiopathic arthritis is characterized by CD30+ T cells directed to human heat-shock protein 60 capable of producing the regulatory cytokine interleukin-10. *Arthritis Rheum* 2003; 48 (7): 2001-10.
 51. de Kleer, IM, Wedderburn LR, Taams LS, Patel A, Varsani H, Klein M, et al. CD4+CD25bright regulatory T-cells actively regulate inflammation in the joints of patients with the remitting form of Juvenile Idiopathic Arthritis. *J Immunol* 2004; in press.
 52. Maurice, MM, Lankester AC, Bezemer AC, Geertsma MF, Tak PP, Breedveld FC, et al. Defective TCR-mediated signaling in synovial T cells in rheumatoid arthritis. *J Immunol* 1997; 159 (6): 2973-78
 53. Black, AP, Bhayani H, Ryder CA, Gardner-Medwin JM and Southwood TR. T-cell activation without proliferation in juvenile idiopathic arthritis. *Arthritis Res* 2002; 4 (3): 177-83.
 54. Patel, A, Varsani H and Wedderburn LR. The hyporesponsiveness of synovial T cells in JIA is a property of CD4+CD25+ T cells : evidence for regulatory T cells in juvenile arthritis. *Rheumatology* 2003; 42 (S1): S11.

55. Cameron, TO, Norris PJ, Patel A, Moulon C, Rosenberg ES, Mellins ED, et al. Labeling antigen-specific CD4(+) T cells with class II MHC oligomers. *J Immunol Methods* 2002; 268 (1): 51-69.
56. Cao, D, Malmstrom V, Baecher-Allan C, Hafler D, Klareskog L and Trollmo C. Isolation and functional characterization of regulatory CD25brightCD4+ T cells from the target organ of patients with rheumatoid arthritis. *Eur J Immunol* 2003; 33 (1): 215-23.
57. Crawley, E, Kon S and Woo P. Hereditary predisposition to low interleukin-10 production in children with extended oligoarticular juvenile idiopathic arthritis. *Rheumatology* 2001; 40 (5): 574-78.
58. Thomson, W, Barrett JH, Donn R, Pepper L, Kennedy LJ, Ollier WE, et al. Juvenile idiopathic arthritis classified by the ILAR criteria: HLA associations in UK patients. *Rheumatology* 2002; 41 (10): 1183-9.
59. von Herrath, M and Homann D. Introducing baselines for therapeutic use of regulatory T cells and cytokines in autoimmunity. *Trends Immunol* 2003; 24 (10): 540-5.

Figure 1

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Legend to figure 1: Flow cytometric analysis of (A) blood and (B, C) synovial fluid T cells, after stimulation assay in vitro. Cells were labelled with the green dye carboxyfluorescein diacetate succinimidyl ester (CFSE), and then cultured in the presence of influenza haemagglutinin peptide (HA307-319) for 5 days. Cells were stained and analysed by flow cytometry. In A and B CFSE-labelled blood or synovial T cells (gated on CD3⁺) are shown stained for CD4, with CFSE on the x axis: those CD4 cells which have divided are in the upper left quadrant. In plot C, CFSE labelled synovial CD4⁺ cells, (gated on CD3⁺CD4⁺), are stained with HA/DR1 tetramer. All cells which have divided and are specific for HA peptide are in the upper left quadrant. Note the population of divided cells which are tetramer negative (i.e. presumed to be antigen non-specific), seen in the lower left quadrant of plot C.