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Review

T cell regulation of the immune response to infection in periodontal diseases

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Summary. Although T cells have been implicated in the pathogenesis and are considered to be central both in progression and control of the chronic inflammatory periodontal diseases, the precise contribution of T cells to the regulation of tissue destruction has not been fully elucidated. Current dogma suggests that immunity to infection is controlled by distinct T helper 1 (Th1) and T helper 2 (Th2) subsets of T cells classified on the basis of their cytokine profile. Further, a subset of T cells with immunosuppressive function and cytokine profile distinct from Th1 or Th2 has been described and designated as regulatory T cells. Although these regulatory T cells have been considered to maintain self-tolerance resulting in the suppression of auto-immune responses, recent data suggest that these cells may also play a role in preventing infection-induced immunopathology. In this review, the role of functional and regulatory T cells in chronic inflammatory periodontal diseases will be summarized. This should not only provide an insight into the relationship between the immune response to periodontopathic bacteria and disease but should also highlight areas of development for potentially new therapeutic modalities.

Key words: Periodontitis, Gingivitis, T cell, cytokine, Immunoregulation

Introduction

Chronic inflammatory periodontal disease can be classified into at least two distinct entities based on immunohistological findings. One is dominated by T cells and clinically corresponds to gingivitis. The other is the B-cell/plasma cell lesion which is seen in chronic periodontitis. Although most children with deciduous teeth suffer from gingivitis, their periodontal ligament and alveolar bone are usually intact. In adults, irrespective of their age, gingivitis also develops soon after abstention from oral hygiene procedures (Fransson et al., 1996), but not all gingivitis lesions proceed to periodontitis.

Chronic periodontitis is a destructive disease that affects the supporting structures of the teeth including periodontal ligament, cementum, and alveolar bone. Some forms of periodontitis are extremely destructive and patients will lose multiple teeth if left untreated (Hirschfeld and Wasserman, 1978). The process of periodontal tissue destruction is considered to be as a result of repeated bursts of activity followed by subsequent silent stable periods within a relatively short time frame (Socransky et al., 1984). Some patients may encounter these bursts of disease activity often while others do not. Because of the predominant lymphocytic infiltration in the periodontitis lesion (Seymour et al., 1979), much effort has been put into elucidating the relationship between lymphocyte function and disease activity as well as in identifying patient susceptibility. Elucidating such mechanisms may facilitate further understanding of the pathogenesis and in so doing help to develop new therapeutic modalities for chronic inflammatory periodontal diseases.

Cytokine profile of T cells in the gingivitis and periodontitis lesions

In 1986, Mosmann et al. first demonstrated that mouse T cell clones can be classified into distinct functional subsets based on their cytokine profile; these were called T helper 1 (Th1) and T helper 2 (Th2) (Mosmann et al., 1986). Since the type of immune response, namely cell-mediated immunity and humoral immunity was determined by Th1 and Th2 cells respectively, it became clear that the response to infection could also be determined by these functional T-cell subsets.

Over the last decade, a number of theories have been postulated to explain the progression of gingivitis to periodontitis in the context of the Th1/Th2 paradigm. There are only a few reports concerning the cytokine

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profile in gingivitis. Our previous study using cryostat sections of gingivitis tissues showed that both Th1 and Th2 cells existed in gingivitis lesions although their frequencies were significantly lower than those in periodontitis lesions (Yamazaki et al., 1995). There was little difference between gingivitis and periodontitis in the expression of phenotypic markers such as IL-2R, HLA-DR or CD45 isoforms by the infiltrating T cells. (Seymour et al., 1988; Takeuchi et al., 1991; Yamazaki et al., 1993). Since the full activation of T cells require engagement of costimulatory molecules in addition to TCR/CD3 complex and as there are positive and negative costimulatory signals (Grewal and Flavell, 1996; Lenschow et al., 1996), the difference in the cytokine profile between two diseases may be reflected by the differential expression of costimulatory molecules. Alternatively, possible specific inhibitory mechanisms may be operating in the gingivitis lesion. In fact, T-cell lines specific to the periodontopathic bacterium *Porphyromonas gingivalis* (*P. gingivalis*) established from peripheral blood of subjects with gingivitis and periodontitis demonstrated no significant difference in the proportion of IFN-γ-, IL-4- and IL-10-positive cells upon activation (Gemmell et al., 1999, 2002a).

On the other hand, there are many but inconsistent reports on the cytokine profile of T cells in periodontitis. Investigators have not only employed several different methods in these studies but have also analyzed different molecules, such as mRNA and proteins. Whereas IFN-γ is reported to be the predominant cytokine in periodontitis tissue by mRNA analysis (Fujihashi et al., 1996; Okada et al., 1996; Takeichi et al., 2000), we and others have shown that IL-4 and IL-6 are predominant at the protein level (Manhart et al., 1994; Yamazaki et al., 1994, 1995). Increased expression of IL-6 mRNA is also reported (Fujihashi et al., 1993, 1996; Lundqvist et al., 1994; Bickel et al., 2001). Readers are advised to refer to specific review papers regarding Th1/Th2 cytokine profiles in periodontal disease for further information (Mathur and Michalowicz, 1997; Seymour and Gemmell, 2001; Taubman and Kawai, 2001; Gemmell et al., 2002b). Overall, distinct Th1 or Th2 lesions which can be seen in mouse models have not been demonstrated in human periodontitis. Nevertheless, these studies have led to the formulation of several hypotheses where T-cell subsets are associated with periodontitis. In the first model, Th1 cells are associated with the stable lesion while Th2 cells are associated with the progressive lesion. In this model, T cells regulate the production of antibodies which can be either protective or destructive. In susceptible patients, non-protective antibodies fail to neutralize or eliminate periodontopathic bacteria resulting in continuation of the disease. On the other hand, protective antibodies may eliminate the bacteria and hence the lesions do not progress further. This model is consistent with the immunohistological observations that Th2 cells predominate in periodontitis lesions and that tissue destruction is mediated by uncontrolled B-cell production of IL-1 (Seymour and Gemmell, 2001) (Fig. 1, Model A). In the second model, Th1 cells are thought to result in tissue destruction and Th2 cells are thought to be protective. In this model, IFN-γ primarily stimulates monocytes/macrophages to produce proinflammatory cytokines and PGE_2, resulting in a local hyper-inflammatory condition, leading to connective tissue destruction and alveolar bone resorption in susceptible patients. However, this model is based largely on animal experiments and a recent report has shown that there is no increase in macrophage numbers and little evidence of macrophage activation in

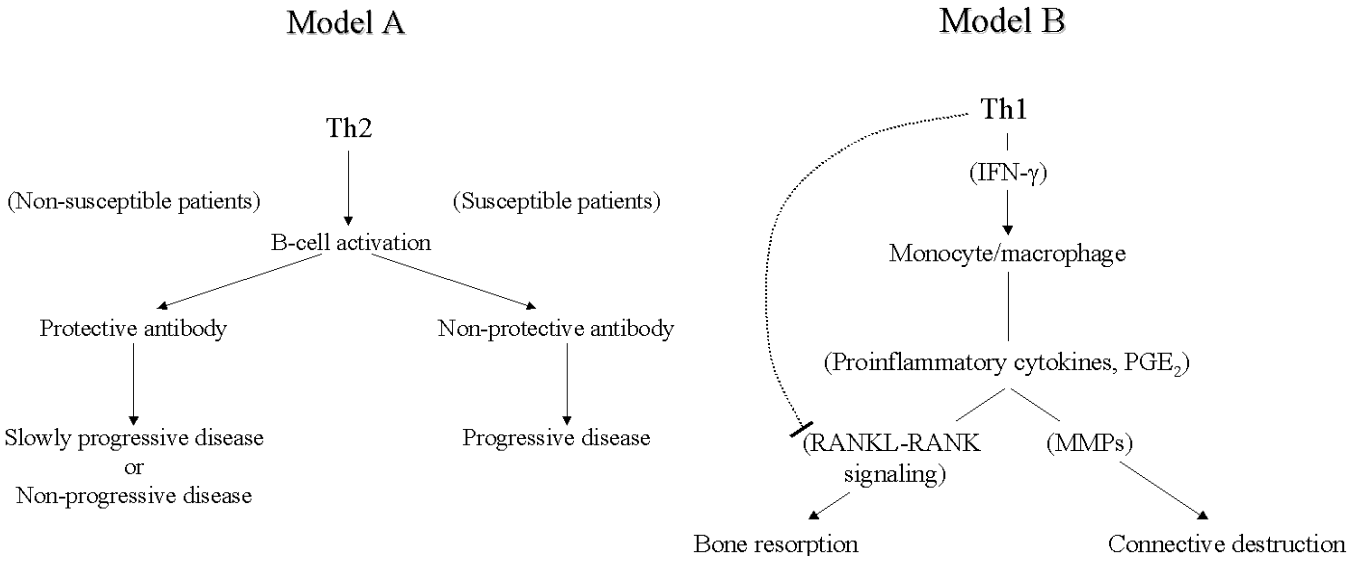

Fig. 1. Models for the role of Th1 and Th2 cytokines in periodontal conditions modified from the models originally appearing in the reference by Gemmell et al. (2002).

advanced periodontitis compared with minimally inflamed tissues (Chapple et al., 1998). Moreover, it has been shown that IFN-γ inhibits osteoclastogenesis by interfering with the RNAKL-RANK signaling pathway (Takayanagi et al., 2000). Therefore, it is also possible that IFN-γ can be a protective cytokine in bone resorption (Fig. 2, Model B). Yet, another controversial model is derived from the alteration of monocyte function by IL-4. IL-4 is considered to function as an anti-inflammatory cytokine by; (i) induction of apoptosis of activated macrophages expressing IL-4 receptor (Yamamoto et al., 1996); (ii) down-regulating CD14 expression by LPS-stimulated monocytes (Lauener et al., 1990); and (iii) suppression of Th1 cytokine production (Abbas et al., 1996). Based on the findings of an absence of IL-4 mRNA in periodontitis lesions but with the presence of macrophages expressing high levels of IL-4 receptor mRNA, it was postulated that a lack of down-regulation of monocytes by IL-4 leads to tissue destruction (Yamamoto et al., 1996). However, the level of IL-13 showing similar biological activities to IL-4 in many aspects is reported to be elevated in periodontitis lesions (Fujihashi et al., 1996; Yamazaki et al., 1997). It is conceivable that IL-13 substitutes the IL-4 function in the lesion. This model is also inconsistent with direct immunohistological evidence of high levels of IL-4-positive Th2 cells in periodontitis.

As described above each hypothesis is weakened by contradicting findings. This is because of uncertainties regarding the presence of ongoing disease activity, the specific microorganisms involved, and other undefined immune parameters. Further studies are clearly required to clarify the role of T-cell cytokines in disease activity and associated immune cell functions.

Auto-reactive T cells and regulatory mechanisms

While the importance of T cells in periodontal disease appears clear, their antigen specificity and function remain obscure. It is clear that periodontopathic

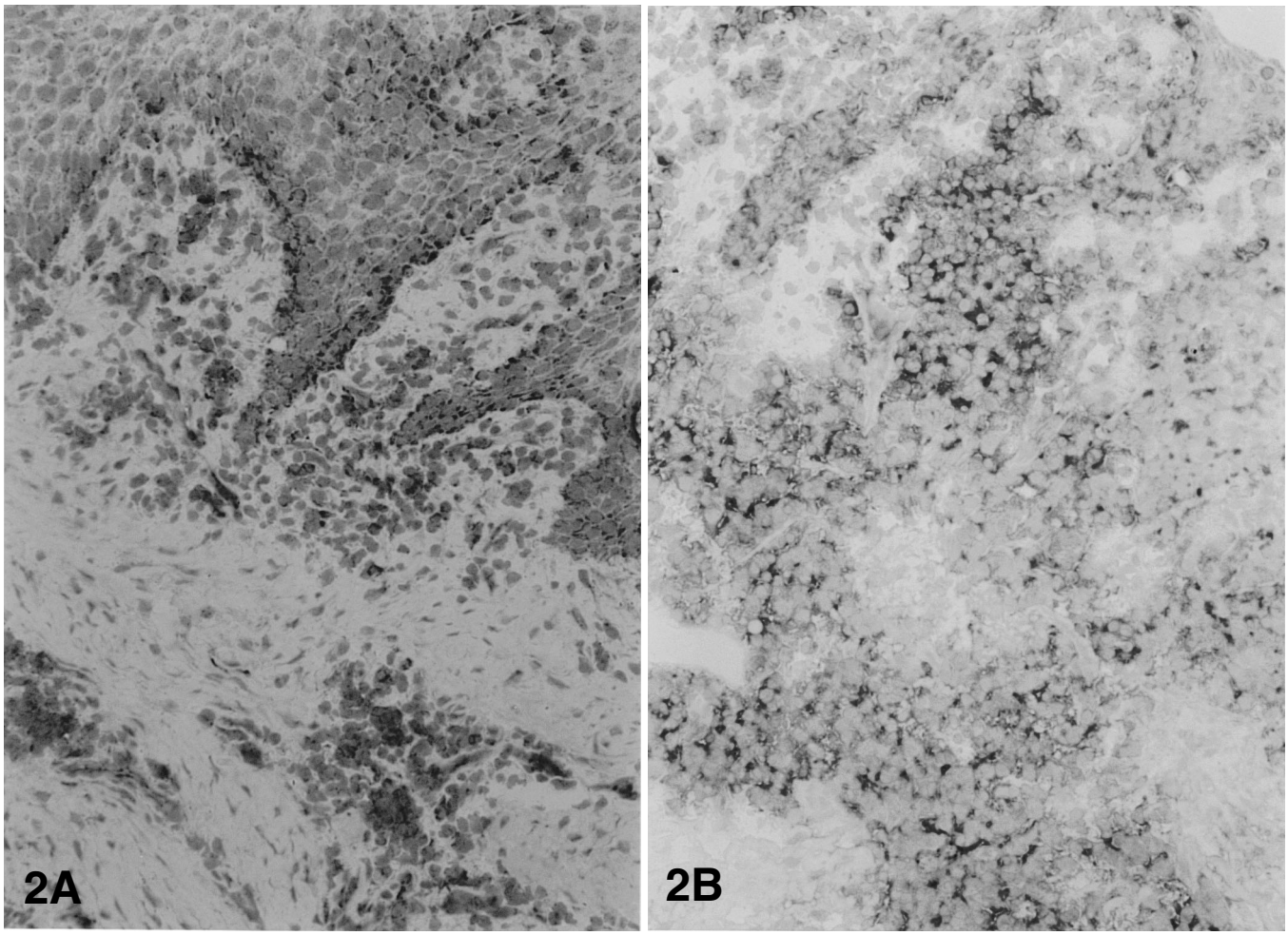

Fig. 2. Expression of HSP60 in periodontitis tissues. Staining of HSP60 was carried out by using an alkaline-phosphatase anti-alkaline-phosphatase (APAAP) method on cryostat sections. HSP60-expressing cells appear as blue. Anti-HSP60 antibody reacts with either basal cells of the oral epithelium **(A)** or cells in the inflammatory infiltrate **(B)**. x 50

bacteria are the primary causative agents. However, there is increasing evidence to suggest that the immune response to self-components is also important in the disease process. Immune responses to self-antigens such as collagen type I, a major component of the periodontium, have been considered for many years as one of the pathogenic pathways following the finding of high titers of anti-collagen type I antibody in the sera of periodontitis patients (Hirsch et al., 1988). It has also been demonstrated that $CD5^+$ B cells, which are supposed to produce auto-reactive antibody, not only increase in periodontitis lesions but also produce more anti-collagen type I antibody *in vitro* than $CD5^-$ B cells (Sugawara et al., 1992). Another auto-reactive molecule candidate is heat shock protein 60 (HSP60). Despite being highly homologous between prokaryotic and eukaryotic cells, HSP60s are strongly immunogenic, and the immune response to microbial HSP60s are speculated to initiate chronic inflammatory diseases in which autoimmune responses to human HSP60 may be central to pathogenesis. It has been reported that GroEL-like proteins belonging to the HSP60 family can be expressed by periodontopathic bacteria (Hotokezaka et al., 1994; Maeda et al., 1994; Nakano et al., 1995; Vayssier et al., 1994). In addition to antibodies to bacterial HSP60, elevated anti-human HSP60 antibody can be found in the sera of periodontitis patients. Furthermore, these antibodies showed cross-reactivity between human HSP60 and *P. gingivalis* GroEL (Tabeta et al., 2000). The expression of HSP60 has also been observed directly in periodontitis lesions (Fig. 2) (Ueki et al., 2002).

Compared with the antibody response, the T-cell response to auto-antigens in periodontitis is less well characterized. Wassenaar et al. (1995) established and characterized T-cell clones from inflamed gingival tissue of periodontitis patients. Whereas 80% of T-cell clones specific to collagen type I were Th2, producing high levels of IL-4 and low levels of IFN-γ, the majority of clones specific to bacterial antigen showed a Th0-profile, producing equal amounts of IL-4 and IFN-γ (Wassenaar et al., 1995). Recently, we examined the proliferative response of PBMC as well as the cytokine profile and clonality of T cells from periodontitis patients and controls, following stimulation with recombinant human HSP60 and *P. gingivalis* GroEL. PBMC from periodontitis patients demonstrated significantly higher proliferative responses to human HSP60 and a much lower response to *P. gingivalis* GroEL compared with control subjects. The response was inhibited by anti-MHC class II antibodies suggesting that it was an antigen-specific response. Specific T-cell responses following stimulation with HSP60 were also demonstrated by RT-PCR-SSCP analysis of the CDR3 region of the T-cell receptor (TCR) ß-chain. Analyses of the nucleotide sequences of the TCR demonstrated that human HSP60-reactive T-cell clones and periodontitis lesion-infiltrating T cells have the same receptors suggesting that HSP60-reactive T cells accumulate in periodontitis lesions. Analysis of the cytokine profile demonstrated that HSP60-reactive PBMC produced significant levels of IFN-γ in periodontitis patients, whereas *P. gingivalis* GroEL did not induce any skewing towards type1 or type2 cytokine profiles. In control subjects no significant expression of IFN-γ or IL-4 was induced. These results suggest that in periodontitis patients, infection with periodontopathic bacteria may induce the activation of self HSP60-reactive T cells with a type1 cytokine profile in an antigen-specific manner (Yamazaki et al., 2002).

Whereas the cytokine profile of collagen type I-reactive T-cell clones is Th2, that of human HSP60-reactive T cells is Th1. The fact that different antigens induce distinct functional T-cell subsets further complicates investigating the role of T cells in the context of cytokine profiles in periodontal diseases.

Regulatory T cells

While it is clear that immunity to infection involves distinct Th1 and Th2 subpopulations of T cells, a further subtype of T cells, with immunosuppressive function and cytokine profiles distinct from either Th1 or Th2 has also been described. Several subsets of regulatory T cells (Tr) with distinct phenotypes and distinct mechanisms of action have now been clarified. However, a great deal of uncertainty remains about the lineages, differentiation factors, antigen specificity and mechanisms of action of these so-called Tr cells. Whether or not these are the exact regulatory T cells in periodontitis remains unclear.

Early studies showed that cells extracted from periodontitis lesions failed to respond, in terms of blastogenesis, to further stimulation *in vitro*, in contrast to peripheral blood lymphocytes (Ivanyi, 1980; Seymour et al., 1985). These data were considered to reflect a possible imbalance in local immunoregulation which may not be present systemically and which may not exist in the gingivitis lesion. Some researchers considered that the increased proportion of $CD8^+$ T cells in periodontitis lesions was a possible mechanism for the impaired proliferative response. However, there is now compelling evidence that $CD4^+$ T cells, which specialize in the suppression of immune responses, play a critical role in immune regulation. The recent finding that cells with this function are enriched within the $CD4^+CD25^+$ subset has prompted a resurgence of interest in T cell-mediated suppression (Asano et al., 1996; Takahashi et al., 1998; Thornton and Shevach, 1998).

As CD25 was initially used as a marker for activated T cells, there are several reports where this antigen was used in the phenotypic analysis of T cells in various forms of periodontal disease. However, it is now conceivable that the $CD4^+CD25^+$ T cell population in these lesions was in fact Tr cells and not recently activated T cells. The proportion of $CD25^+$ subsets within $CD4^+$ T cells appeared to range from 6% to 8% (Dieckmann et al., 2001; Taams et al., 2001). Seymour et al. (1988) reported that less than 10% of T cells expressed IL-2 receptor (CD25) at any stage during the 21-day experimental gingivitis period. However, they

did not show the actual proportion of $CD25^+$ cells within the $CD4^+$ T cell subset. Takeuchi et al. (1991) examined CD25 expression on lymphocytes extracted from gingival tissues from periodontitis patients. The proportion of CD25-positive cells within the $CD4^+$ T cell population in gingival tissue and peripheral blood was 18.4±4.4% and 13.7±7.3%, respectively. The difference between gingival tissue and peripheral blood was statistically significant. A characteristic feature of $CD4^+CD25^+$ Tr cells is that these cells fail to proliferate and to secrete IL-2 in response to TCR ligation but are able to suppress the responses of $CD4^+CD25^-$ cells in co-culture in a cell contact-dependent manner (Thornton and Shevach, 1998).

Recent *in vivo* studies suggest that the function of $CD4^+CD25^+$ T cells is crucially dependent on signaling via the cytotoxic T lymphocyte-associated antigen (CTLA)-4 molecule which was found to be constitutively expressed on $CD4^+CD25^+$ T cells (Read et al., 2000; Salomon et al., 2000; Takahashi et al., 2000). Blockage of CTLA-4 inhibits the function of Tr cells *in vitro*, even under circumstances in which CTLA-4 is present only on the Tr cell population. This suggests that CTLA-4 expression on $CD4^+CD25^+$ T cells is involved in their function. However, since others have found that *in vitro* suppression cannot be abrogated by blockage of CTLA-4 (Baecher-Allan et al., 2001), how CTLA-4 is involved in the function of Tr cells remains to be defined. Recently, we examined CTLA-4 expression by peripheral blood $CD4^+$ T cells following stimulation with the *P. gingivalis* outer membrane protein (OM) (Aoyagi et al., 2000). In periodontitis patients, CTLA-4 expression was up-regulated with concomitant elevation of CD25 expression following stimulation with *P. gingivalis* OM and to a lesser extent with immobilized anti-CD3 antibody. The percentages of CTLA-4-expressing $CD4^+$ T cells stimulated with *P. gingivalis* OM and anti-CD3 were 34.4% and 9.6%, respectively. When compared between periodontitis patients and periodontally healthy control subjects, the percentage of CTLA-4-expressing $CD4^+$ T cells was higher in periodontitis patients than in control subjects. Interestingly, *P. gingivalis* OM stimulation did not enhance an antigen-specific secondary response. This suggests that increased Tr in the initial response might be involved in suppressing a secondary response. Although actual expression of CTLA-4 on $CD4^+CD25^+$ T cells was not examined in this experiment, these data might suggest that $CD4^+CD25^+$ Tr cells play a role in controlling auto-reactive T cells activated during the immune response to periodontopathic bacteria. On the other hand, Gemmell et al. (2001) examined CTLA-4-positive cells in gingival biopsies from healthy/gingivitis and periodontitis subjects and demonstrated that T cells express CD28 rather than CTLA-4 in both healthy/gingivitis and periodontitis tissue sections and that the percentage of CTLA-4-positive cells remained low. They concluded that T cells in both healthy/gingivitis and periodontitis tissues are not regulated negatively by CTLA-4. Although the discrepancy between these investigations has not been resolved, one important question that must be addressed is the nature of the physiological ligand recognized by the TCR expressed on $CD25^+$ T cells.

Natural killer T cells

Recently, a unique lymphocyte population designated natural killer T cells (NK T cells), has been characterized. NK T cells express common markers for NK cells and the invariant Vα-Jα TCR (Lantz and Bendelac, 1994; Bendelac, 1995; 1997; Bix and Locksley, 1995; MacDonald, 1995; Vicali and Zlotnik, 1996). Human invariant Vα24JαQ T cells are homologous to the murine Vα14Jα281 NK 1.1^+ T cells. These cells have a TCR α chain in which the Vα14 segment is rearranged to pair with Jα281 with no N-region diversity (Dellabona et al., 1994; Exley et al., 1997; Porcelli et al., 1993). These NK T cells have functionally important roles *in vivo*. A direct relationship exists between a deficiency in NK T cells and susceptibility to type 1 diabetes in nonobese diabetic (NOD) mice (Baxter et al., 1997; Godfrey et al., 1997; Gombert et al., 1996; Hammond et al., 1998), and in humans (Wilson et al., 1998). A deficiency in NK T cells has also been implicated in some other autoimmune diseases including autoimmune gastritis (Hammond et al., 1998) and lupus-like disease (Mieza et al., 1996; Takeda and Dennert, 1993) in mice and in humans with systemic sclerosis (Sumida et al., 1995). These studies suggest a role for NK T cells in the regulation of autoimmune diseases. We investigated the proportion of the invariant Vα24JαQ TCR within the Vα24 T cell population in periodontitis lesions and gingivitis lesions using SSCP methodology (Yamazaki et al., 2001). NK T cells were identified with a specific JαQ probe whereas the total Vα24 TCR was identified using an internal Cα probe. NK T cells were a significant proportion of the total Vα24 population both in periodontitis lesions and to a lesser extent in gingivitis lesions, but not in the peripheral blood of either periodontitis patients or non-diseased control subjects. Using immunohistochemistry, some of $V\alpha24^+$ cells in the periodontitis lesions seemed to associate with $CD1d^+$ cells, which are specific antigen-presenting cells for NK T cells. Although the mechanism underlying the elevation of NK T cells in both periodontitis and gingivitis lesions remains unclear, it can be postulated that NK T cells are recruited to play a regulatory role in the auto-immune response generated by the bacterial infection. The mechanisms through which NK T cells may regulate autoimmunity remain unclear. When activated through their TCR, NK T cells become cytotoxic and produce a variety of cytokines, including IL-4, IFN-γ, IL-10 and TGF-ß (D' Orazio and Niederkorn, 1998). As these cytokines are produced by activated $CD4^+CD25^+$-regulatory T cells, further investigations are needed to determine how these cytokines are involved in the protection against autoimmune-mediated tissue destruction in periodontal

diseases.

Therapeutic implications of T cell analysis in periodontal diseases

Involvement of T cells in the immunoregulation of periodontal disease is now well established. It is becoming clear that the clinical manifestations of periodontitis may be associated with autoimmune mechanisms in which there is activation of a dominant, self-reactive T cell population but also, failure of the peripheral immune regulatory mechanisms that normally control these cells. One important regulatory mechanism is centered on the recognition of TCR-derived peptides in the context of Class I and class II MHC by regulatory $CD8^+$ and $CD4^+$ Tr cells, respectively.

The ability to induce or expand Tr cells *in vivo* and *in vitro* could have important implications in patients with these forms of periodontitis in which auto-immune mechanisms are involved. An advantage is that because Tr cells can exert bystander suppression in an antigen-nonspecific manner, they need not necessarily recognize the target antigen(s) that are the subject of the auto-immune response. Induction of Tr cells that react to any local tissue-expressed molecule may be sufficient to inhibit immune pathology. Identification of the sites of action of Tr cells and of their antigen reactivities will be paramount in applying this kind of strategy to the treatment of inflammatory diseases. One important area that remains to be addressed is whether Tr cells can also down-regulate an ongoing immunopathological reaction. If so, what manipulations are required to re-establish dominant Tr cell activity in vivo? In addition, identification of downstream cellular targets and molecular mechanisms of Tr cell action should further enhance the development of treatments that inhibit immune pathology. Manipulation of Tr cells may also have important clinical benefits in the induction of protective immunity.

NK T cells associate with CD1d-expressing dendritic cells (Bendelac et al., 1997). The TCR of NKT cell recognizes a conserved family of glycolipids. It has been well established that virtually all mouse and human NK T cells can recognize α-galactosylceramide (α-Galcer), a glycolipid originally extracted from marine sponges. Although the mammalian counterpart of α-Galcer has yet to be identified, it is conceivable that it is induced under a range of pathological and inflammatory conditions to activate NK T cells. Nevertheless, identification of T cells reactive with auto-antigens and those with regulatory roles would provide another working hypothesis for the pathogenesis of periodontal disease (Fig. 3).

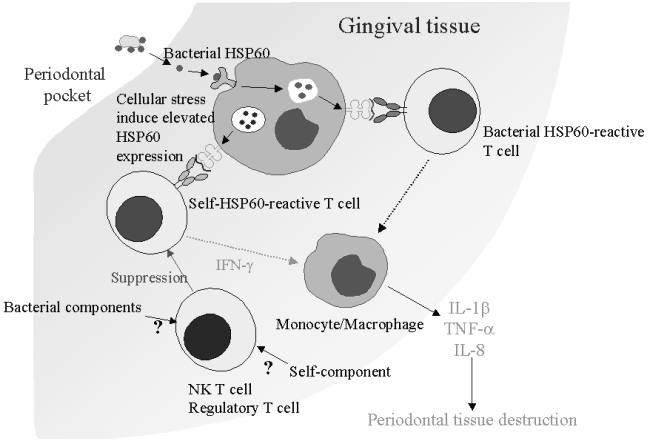

Fig. 3. Infected periodontopathic bacteria are processed by antigen-presenting cells and bacterial HSP60 is also presented and recognized by specific T cells. At the same time inflammation induces up-regulation of HSP60 expression of various host cells, hence self-HSP60-reactive T cells are activated. These T cells may produce Th1 cytokine resulting in the activation of monocyte/macrophage, or produce Th2 cytokine resulting in the production of antibodies specific to bacterial as well as endogenous HSP60. However, regulatory T cells such as NK T cell or $CD4^+CD25^+$ T cells, if appropriately recruited and activated, suppress deteriorating auto immune response.

Conclusion

Accumulating evidence has certainly suggested that the analysis of T cells is of particular importance in not only understanding the pathogenesis of periodontal disease but also in the development of new immunotherapies. However, as with other infectious and auto-immune diseases, immune mechanisms underlying the diseases have been proven to be more complex than previously thought. The number of cytokines and CD antigens are increasing year by year. Nevertheless, we will soon obtain the complete data set in terms of the human genome and subsequently all the functions of every gene will be known. Although removal of bacteria and their components will undoubtedly keep their position in the treatment of periodontal disease, alternative treatments based on the understanding of the biological process should come of age.

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