Susceptibility to periodontitis. Studies with LPS-stimulated whole blood cell cultures

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CHAPTER 7

The Central Role of Monocytes in Systemic Immune Effects
Induced by the Chronic Periodontal Infection

General Discussion
LPS-stimulated whole blood cell cultures reflect the behavior of monocytes

Periodontal disease is a multifactorial infectious entity, affecting the tooth supporting tissues and eventually, when not treated, resulting in overall teeth loss (1). Not only environmental factors, such as smoking and periodontal pathogens, play an important role (2, 3), but also the genetic make-up of the host is crucial (4-6). Despite the fact that microorganisms, colonizing the tooth surfaces, are essential in the induction of the periodontal inflammation, not everyone develops a severe form of destructive periodontal disease (7). Therefore, not everyone is equally susceptible to periodontal disease and the host immune response is of fundamental importance (1, 8).

In order to study the host immune response, WBCC stimulated with LPS derived from E. coli were used in the research papers, as presented in the current thesis. In response to LPS, CD14 is required together with the accessory protein MD-2 and the LPS-binding protein (9-13). Subsequently, LPS is presented to Toll-like receptor (TLR)-4 to induce signal transduction, since CD14, the principal receptor for LPS on myeloid cells, lacks a cytoplasmic domain (14). Recently, it has been clearly demonstrated that CD14 and TLR-4 are highly expressed by peripheral blood monocytes, and that the monocyte is the key orchestrator of LPS responsiveness in WBCC (15). In addition, previous studies and experiments revealed that LPS-stimulated WBCC specifically reflect the behavior of the monocytes when relatively low concentrations of LPS are used (16-18). Furthermore, when monocytes are isolated from the peripheral blood and stimulated with LPS, cytokine production is modulated in the same manner as in LPS-stimulated WBCC (18-21). However, in isolated monocytes the production of the cytokine IL-12 is importantly diminished, due to the induction of an inhibitory, as yet unknown, component upon purification of the cells (17, 22).

Upon LPS stimulation the synthesis of inflammatory mediators, such as cytokines, chemokines and prostaglandins, is induced in the monocytes (23). The stimulated chemokine expression is essential in directing the peripheral blood monocytes and other leukocytes in the emigration from the circulation into the site of infection, in
case of periodontitis into the periodontal lesion (24). Within the periodontal lesion chemokines, like MCP-1 and IL-8, are highly expressed, causing a gradient across the endothelium and facilitating the transmigration of peripheral monocytes and neutrophils respectively (25-28). Crossing the endothelial tissues the monocytes start to differentiate into either macrophages, important scavengers in the inflamed periodontal lesion, or immature DCs (29). Subsequent maturation of DCs in the periodontal lesion occurs upon activation by pathogens or their products and by inflammatory mediators derived from the local micro-environment (30-32). The matured DCs carry this information to the draining lymph nodes and instruct the cells of the specific immunity by eliciting different classes of Th cell responses, i.e. Th1 and Th2. This differentiation of specific Th responses is not only determined by the type of pathogen (33, 34), but also by the type of tissue that is invaded (32, 35, 36). This indicates that the local environment, e.g. inflammatory mediators, instructs immature DCs, and in response to that DCs have the ability to adopt reciprocally polarized Th1- and Th2-promoting phenotypes (37). This plasticity of DC function is however lost upon maturation (38). Since the similar plasticity has been observed for peripheral blood monocytes (Kaliński, unpublished data) as for monocyte-derived DCs (37), it may be suggested that the immature blood-derived precursor cells respond in a functionally flexible way towards changes in their local microenvironment, i.e. the peripheral blood. Therefore, in this way the APCs orchestrate the modulation of the immune response and interface the innate and adaptive immunity (31).

The studies presented in this thesis made use of LPS derived from *E. coli* to stimulate the WBCC. The reasons for using this non-specific stimulant in triggering the production of inflammatory mediators in monocytes, was based on the fact that not all patients and controls were always colonized by the same major periodontal pathogens (chapter 2). If cell wall components of a specific periodontal pathogen were employed, one could argue which pathogen should be used in order to study the susceptibility to periodontal disease, i.e. investigating differences in the host immune response. In this respect, it is proposed that *P. gingivalis* may have a unique
role in the pathogenesis of periodontal disease, since LPS derived from \textit{P. gingivalis} causes an unusual innate host response, i.e. antagonizing endothelial and gingival epithelial cells (reviewed in 39). In addition, LPS from \textit{P. gingivalis} antagonize the human TLR-4 and favor instead the use of TLR-2 (40, 41), whereas the latter receptor is only weakly expressed by endothelial cells (42). The binding of \textit{P. gingivalis} LPS via this TLR-2 has, at least in mice, been shown to induce different classes of specific immune responses most likely through the production of diverse cytokines by a specific functionally distinct subset of DCs (43). However, in humans monocyte-derived DCs triggered with LPS from either \textit{P. gingivalis} or \textit{E. coli} did not show a different cytokine profile, although stimulation with whole bacteria did (44). Despite these characteristics of \textit{P. gingivalis} LPS and its immune consequences, it was shown that \textit{P. gingivalis}-positive subjects suffering from periodontitis and \textit{P. gingivalis}-positive control subjects without destructive periodontal disease elicited highly variable cytokine profiles in their \textit{P. gingivalis}-specific T cell clones (45, 46). However, the clones from patients displayed more variation and a greater number of patients showed a different cytokine profile than controls. Consequently, it was suggested that the nature of the antigen not necessarily selects for a particular cytokine profile \textit{in vivo} and that immune differences may be due to host susceptibility rather than the specific antigen (47). Finally, \textit{E. coli}-derived LPS was used to activate the monocytes in the WBCC, since many studies have used this common available and highly purified source of LPS (16, 17, 48-51), and therefore it facilitates comparisons with other studies within the field of periodontology and in other areas of research concerning distinct inflammatory diseases.

\textbf{Monocyte response in periodontal health and disease}

When in the late 80's of the previous century the LPS-stimulated responses in peripheral blood monocytes from periodontitis patients were studied for the first time, the reasons for this were based on the LPS unresponsiveness of C3H/HeJ mice (52). Since periodontitis is associated with the colonization of predominantly Gram-
negative bacteria, it was postulated that patients susceptible to destructive periodontal disease were, like these mice, not able to respond properly to LPS. As is known nowadays, C3H/HeJ mice do not respond to LPS derived from enterobacteria due to a point mutation in TLR-4 (53, 54). Interestingly, Garrison and Nichols (1989) stimulated peripheral blood monocytes from periodontitis patients with LPS derived from different periodontal pathogens, and found a 2- to 3-fold higher release of PGE\(_2\) by patients compared to control subjects. This difference was observed for all used LPS preparations, including \(P.\ gingivalis\) that is nowadays known to agonize TLR-2 (40, 41). The increased PGE\(_2\) release by the peripheral blood monocytes from periodontitis patients was interpreted as a hypersecretory trait of the patients' monocytes, and this trait was believed to be responsible for the susceptibility to periodontal destruction. Especially, since this trait highly correlated with enhanced PGE\(_2\) levels in the periodontal pocket, and in addition, these locally enhanced levels corresponded with the clinical severity of the disease (55). This concept of hypersecretory monocytes from periodontitis susceptible patients, releasing enhanced levels of PGE\(_2\), and its role in the pathology of the disease was additionally strengthened by the fact that PGE\(_2\) stimulates osteoclastic bone resorption, one of the main characteristics of periodontitis (56). Above that, non-steroidal anti-inflammatory drugs, inhibitors of PGE\(_2\) production, were shown to inhibit the loss of alveolar bone in periodontitis (57-59).

Therefore, based on the concept of hypersecretory monocytes, several other studies in the field of Periodontology, investigated the production of pro-inflammatory mediators, i.e. PGE\(_2\), IL-1\(\beta\), TNF-\(\alpha\) and IL-6, by peripheral blood monocytes from patients using either periodontal pathogen- or \(E.\ coli\)-derived LPS sources. However, the data of these studies revealed conflicting results (48, 60-62). These differences may be explained by improper experimental design: inconsistent and different inclusion criteria for the patient populations, and even the absence of disease-free control groups. According to proper inclusion criteria, there is only one study making true comparisons possible with the data presented in the current thesis. In that
specific study isolated peripheral blood monocytes were used and stimulated with LPS from *E. coli* (48). Since the data of that study corroborate the present results using WBCC that were also stimulated with *E. coli* (chapter 2), it confirms that the LPS-stimulated cytokine production by isolated peripheral blood monocytes from periodontitis patients is modulated in the same manner as in LPS-stimulated WBCC. However, all previous studies concerning the LPS responsiveness of peripheral blood monocytes only analyzed the production of common pro-inflammatory mediators, such as PGE$_2$, IL-1$\beta$ and TNF-$\alpha$, and interpreted their potential roles in destructive periodontal disease exclusively as mediators of alveolar bone destruction. Neither the monocyte-derived release of chemokines, anti-inflammatory and immune-regulatory cytokines were investigated before, nor such features were highlighted in the pathogenesis of destructive periodontal disease.

There was no difference between periodontitis patients and control subjects in the release of the major pro- and anti-inflammatory cytokines, i.e. TNF-$\alpha$, IL-6 (48) and IL-10, indicating that there is not a general failure in APCs from periodontitis patients to produce cytokines (chapter 2). However, the release of the pro-inflammatory cytokine IL-1$\beta$ was somewhat lower in patients (48, chapter 2), suggesting a depressed cellular immunity in periodontitis patients. The APC product IL-1$\beta$ has been shown to participate in the priming of human Th lymphocytes and co-stimulates Th cell responses by up-regulating the IFN-$\gamma$ production (63). Furthermore, a decreased IL-1$\beta$ production has been correlated with a reduced T cell proliferation in atopic dermatitis patients (64, 65). The WBCC as well as the isolated monocytes from the periodontitis patients both stimulated with LPS from *E. coli*, released on average 2 to 3 times higher levels of PGE$_2$ compared to control subjects (48, chapter 2). Reciprocally, the WBCC from patients released lower levels of IL-12p70 (the biologically active heterodimer of IL-12), while the levels of IL-12p40 did not differ (chapter 2). PGE$_2$ is known to exert a Th2-promoting activity either directly by inhibiting the Th cell production of IFN-$\gamma$ (66, 67) or via an IL-12-antagonistic way by suppressing the production of IL-12p70 (16, 68), while IL-12p70 is obligatory for the
induction of Th1 cell responses (69, 33). Therefore, it was proposed that APCs from periodontitis patients have a Th2-promoting phenotype and in that way might support the humoral immunity in the disease (chapter 2). Indeed, the number of Th2 cells expressing high levels of IL-4 and IL-5 is increased in the periodontal lesion (70-74) and due to their production of B cell growth and differentiation factors (33) the lesion is dominated by B cells and plasma cells (reviewed in 25). Interestingly, consistent with the observed Th2-promoting phenotype of APCs is the depressed Th1 cell response in periodontitis (75, 76): Stimulated peripheral blood mononuclear cells (PBMCs) from periodontitis patients showed decreased proliferative responses and decreased production of IFN-γ, compared to control subjects without destructive periodontal disease. Earlier studies already revealed a depressed cellular response in periodontitis using the autologous mixed lymphocyte response (77, 78). However, these reported depressed cellular immune responses in periodontitis are very likely not a defect in the T cells, but are due to PGE₂ released by the simultaneously present monocytes in these stimulated PBMC cultures (79-83). This Th2-promoting phenotype of APCs and the consequently decreased Th1 cell response in PBMCs have also been found for other chronic inflammatory diseases, like atopic dermatitis, allergic asthma and rheumatoid arthritis, and HIV infection (17, 51, 79-87).

In the LPS-stimulated WBCC the release of the major CXC and several CC chemokines was also analyzed in the present thesis. The cultures of the periodontitis patients released higher levels of the CXC chemokine IL-8 and RANTES in comparison to control subjects, and in addition a trend towards enhanced MCP-1 levels. Concomitant release of MIP-1α, MIP-1β and MDC did not differ between patients and controls (chapters 2 and 4). Since the CXC chemokine IL-8 and the CC chemokine MCP-1 are belonging to the first line of defense molecules, recruiting neutrophils and monocytes respectively to the site of inflammation (88), the higher expression by peripheral blood monocytes in periodontitis patients may reflect a higher activity state of the immune system. Apart from chemo-attracting immune cells, chemokines also activate these cells, whereas CC chemokines have even
important direct and indirect effects on the differentiation of Th cells (reviewed in 89). In this respect, it has been shown that MCP-1 is able to promote the development of Th2 cell responses (90), either directly through enhancing the secretion of IL-4 by Th cells (91, 92) or indirectly through the inhibition of the Th1 skewing cytokine IL-12p70 in APCs (93, 94). Furthermore, it appeared that the monocyte-derived levels of MCP-1 in the LPS-stimulated WBCC from periodontitis patients correlated in a consistent and inversely way with the IL-12p70 levels (chapter 4). This suggests a MCP-1 regulated suppression, most likely in an autocrine or paracrine way, of the IL-12p70 production in the monocytes from patients. In this way, the enhanced MCP-1 expression in periodontitis patients contributes to the Th2-promoting phenotype of their APCs, and might subsequently support the humoral immunity in the disease process. In addition to the role of MCP-1 in T cell differentiation, in vitro experiments have shown that the CC chemokine RANTES can actually induce T cell proliferation and activation in a mitogen-like manner through its aggregation on the cell surface (95, 96). This T cell activation correlates with the expression of the T cell receptor and induces the up-regulation of co-stimulatory molecules and hence cytokine production (96-98). These data on the specific behavior of RANTES suggest an antigen-independent activation of Th cells by the chemokine and, consequently, the enhanced levels of RANTES in periodontitis patients may ameliorate the promotion of Th2 cell responses.

**Monocyte response after therapy**

The bias of peripheral blood monocytes from periodontitis patients to promote Th2 cell responses, and the consequently induced aberrant function of the peripheral blood lymphocytes towards decreased Th1 responses, might be an intrinsic or acquired characteristic of the monocytes. In order to explore whether this bias of APCs from periodontitis patients is derived from the chronic periodontal inflammation, releasing danger molecules in the peripheral blood and thereby causing an altered environment to the functionally flexible monocytes, the effect of disease elimination
should be analyzed. Generally, some chronic inflammatory disorders are treated by the use of topical or either systemically administered anti-inflammatory and immuno-suppressive drugs for extended periods of time or even lifetime. Such drugs will not only affect the local host immune responses but the systemic responses as well; in this respect, glucocorticoids have been shown to strongly inhibit the production of the Th1-polarizing factor IL-12p70 in immature monocyte-derived dendritic cells in a permanent way (99, 100). In contrast, periodontitis is treated by (surgical) mechanical procedures and sometimes in addition of short-term used systemic antibiotics. Since bacteria or their components in the dental plaque that accumulates on the surfaces of all the teeth are considered to be the main etiologic factor in periodontitis (101, 102), therapy is aimed at the reduction of the bacterial load. Early studies showed that following non-surgical periodontal therapy, the depressed autologous mixed lymphocyte response returned to within normal limits in periodontitis patients (103, 104). The Th2-promoting capacity of the monocytes from periodontitis patients, as evaluated in LPS-stimulated WBCC, also reversed after extensive non-surgical periodontal therapy including the use of systemic antibiotics (chapters 3 and 4). The levels of IL-12p70 increased strongly 3 months after periodontal therapy, whereas the MCP-1 levels decreased and the PGE$_2$ levels showed a trend towards reduction. In addition to the data presented in chapter 3, on average the ratio of the PGE$_2$ and IL-12p70 concentrations decreased 2 times ($P = 0.03$). As has been shown in the literature, the net IFN-$\gamma$ production of T cells is largely determined by the ratio of APC-derived PGE$_2$ and IL-12p70 at the time of T cell activation (105). The decreased PGE$_2$ to IL-12p70 ratio indicates that the APCs from patients will favor the promotion of Th1 cell responses instead of Th2 responses after periodontal therapy. Also after therapy, the percentage changes in the MCP-1 and IL-12p70 levels consistently correlated in a reversed way, being decreased MCP-1 levels and increased IL-12p70 levels. This pointed out again the close relationship between both cytokines, and suggested a regulatory role for MCP-1 in the reversed Th2-promoting phenotype of patients' APCs after therapy. In addition, the percentage decreases of MCP-1 and
the reciprocal increases of IL-12p70 levels after periodontal therapy, also consistently correlated with the percentage improvement of the clinical periodontal condition following therapy (chapter 4). Moreover, 2 out of 8 treated patients that showed a less favorable treatment result, i.e. having either more residual bleeding upon probing or more deeper probing depths, showed neither a decrease in the levels of MCP-1 nor a strong increase in the IL-12p70 levels. Despite the reversal of the Th2-promoting phenotype of the monocytes, i.e. comparable levels of IL-12p70, MCP-1 and PGE\(_2\) after therapy to those of control subjects without periodontal disease, the enhanced production of RANTES in untreated periodontitis patients remained after therapy (chapter 4). This suggested that the enhanced expression of RANTES by the monocytes from periodontitis patients is rather an intrinsic characteristic of these cells than acquired and therefore may be an important susceptibility factor in the pathogenesis of periodontal disease. Interestingly, 2 genetic polymorphisms for RANTES were recently associated with rheumatoid arthritis and atopic dermatitis (106, 107). For atopic dermatitis the mutation in the promoter of the gene proved to be functional and appeared to be more frequent in individuals from African descent.

Successful periodontal therapy results clinically in shallow probing depths and a low tendency towards bleeding on probing and microbiologically in a low detection level or even an undetectable level of periodontal pathogens (108, chapters 3 and 4). Although, these parameters were importantly decreased in the presented studies, still about 10% of the sites displayed deepened probing depths and 20 to nearly 30% of the sites showed bleeding on probing (chapters 3 and 4). One could argue whether this residual periodontal infection will still have impact on the responsiveness of peripheral blood monocytes. In order to study this question, complete elimination of the periodontal infection should be installed, which, in case of periodontitis, can be achieved by removal of all the teeth. Therefore, a case with generalized terminal adult periodontitis was treated by a full mouth tooth extraction and the LPS-stimulated response of the peripheral blood monocytes was evaluated over a period of 3 years (chapter 5). The study analyzed the changes over time for the production of inflammatory mediators and was able to demonstrate a 2-fold decrease of the IL-8
and MCP-1 levels, whereas other mediators, such as IL-12p70 and PGE$_2$, were not found to change over 3 years of time. Most likely, due to the limitations of the statistical analysis (the experimental material consisted of 1 single case) the reported return to control levels of PGE$_2$ and IL-12p70 in patients 3 months after periodontal therapy could not be detected in this single case report. However, 3 months after the full mouth tooth extraction therapy the IL-12p70 levels were sharply increased. In contrast, the levels of RANTES did not change over a period of 3 years time after the extraction therapy. This corroborates the unaffected levels reported after non-surgical periodontal therapy and supports the concept that the enhanced expression of RANTES by monocytes from periodontitis patients may be an intrinsic characteristic.

**Systemic responses in periodontitis**

In the previous paragraphs the phenotype of the peripheral blood monocytes, as found in patients suffering from destructive periodontal disease but also from other chronic inflammatory diseases, has been extrapolated to the instructive nature of the APCs within the peripheral tissues. This is permitted, since monocytes isolated from peripheral blood and generated into DCs have been shown to display the same aberrant phenotype and resemble the *in vivo* ones (109). Moreover, peripheral blood monocytes secrete the similar cytokine profile as immature monocyte-derived DCs in response to priming factors and both skew towards either a Th1- or Th2-promoting phenotype (Kaliński, unpublished data, 37). When the peripheral blood monocytes cross the endothelium they become DCs (29) that are subsequently activated within the periodontal tissues (110). Therefore, the final phenotype of matured DCs within the periodontal tissues is most likely determined by pathogens, or their products, and cytokines derived from that local tissue microenvironment, i.e. epithelial cells and fibroblasts (30, 31). Due to the high functional flexibility of the immature DCs in response to environmental factors, the definite promotion of the adaptive immunity is decided within the peripheral tissues themselves.
Therefore, the reactivity of peripheral blood monocytes can be interpreted as a systemic response to exogenous and endogenous danger molecules released by the periodontal infection. Recently, it has been shown that periodontitis results in bacterial endotoxemia, and may cause chronically or even permanently release of pro-inflammatory components into the bloodstream (111). These circulating danger or pro-inflammatory molecules turn on the peripheral blood monocytes, known to be highly responsive and secretory cells. As has been stated for patients suffering from rheumatoid arthritis, their peripheral blood monocytes release increased levels of IL-8, indicating that these cells are activated (112, 113). This similar observation has now been performed for periodontal disease, i.e. patients’ monocytes releasing higher levels of IL-8 compared to controls without destructive disease (chapter 2). The CXC chemokine IL-8 is the major chemo-attractant for neutrophils and represents together with the CC chemokine MCP-1, the major chemo-attractant for monocytes, the first line of defense mediators (88, 114). Since the major source of IL-8 \textit{in vivo} are the monocytes (119) and the IL-8 levels in plasma correlate with the number of monocytes expressing IL-8 mRNA (120), it might be expected that the circulating IL-8 levels in plasma from periodontitis patients are higher compared to control subjects. However, a study (chapter 6) presented in the current thesis did not find differences in the plasma IL-8 levels between periodontitis patients and controls, as did another study (121). This indicates that also in the control group some inflammation, in terms of gingivitis (a superficial non-destructive and reversible inflammatory response of the periodontal tissues), may have been present. In addition, the plasma IL-8 levels in (un)treated periodontitis patients as well as in controls were shown to correlate with the corresponding monocyte-derived IL-8 levels in the LPS-stimulated WBCC (chapter 6). This suggested that IL-8 levels measured in plasma from periodontitis patients are most likely derived from the circulating monocytes.

It is common knowledge that infection in general results in increased numbers of circulating neutrophils (115). Indeed, also the periodontal infection results in increased numbers of peripheral blood neutrophils, and also in experimental gingivitis.
this is the case (116-118). Such an increased number of neutrophils would be expected to parallel the increased monocyte-derived IL-8 production in periodontitis patients. However, no differences in the number of peripheral blood leukocytes or either neutrophils were observed in the same study subjects (chapter 2), which was in line with the equal IL-8 levels in plasma from periodontitis patients and controls (chapter 6). Interestingly, after non-surgical periodontal therapy the number of neutrophils decreased (122, chapter 3), as did the IL-8 levels in plasma (123, chapter 6). Moreover, after complete elimination of the periodontal infection the same reductions were seen, as well as a decreased IL-8 release in the LPS-stimulated WBCC, and even persisted 3 years long (chapters 5 and 6). Altogether, these data suggest that the periodontal infection induces systemic activation of circulating monocytes, reflected by increased IL-8 release upon LPS stimulation, consequently resulting in the recruitment of more neutrophils. In addition, reduction or elimination of the periodontal infection restores the monocyte responsiveness and the number of recruited neutrophils.

For the CC chemokine MCP-1 similar observations have been made. As the peripheral blood monocytes from patients tended to secrete higher MCP-1 levels than controls (chapter 4), the MCP-1 levels in plasma were also higher in patients compared to controls (chapter 6). However, while the monocyte-derived levels of MCP-1 in LPS-stimulated WBCC decreased after non-surgical therapy as well as after full mouth tooth extraction therapy (chapters 4 and 5), the chemokine levels in plasma did not change (chapter 6). It has been shown that MCP-1 is also produced by endothelial and smooth muscle cells and macrophages monocytes in the arterial walls, and therefore the MCP-1 levels detected in plasma are not solely derived from the circulating monocytes (124, 125). Indeed, in contrast to IL-8 the plasma MCP-1 levels did not correlate with the levels secreted by the corresponding LPS-stimulated monocytes (chapter 6). It has been shown that enhanced MCP-1 levels in plasma correlate with increasing age in healthy humans, suggesting to reflect the degree of atherosclerosis (126). Indeed, MCP-1 is hardly found in normal arterial walls, whereas enhanced levels are present in human atherosclerotic plaques (124, 127,
128). MCP-1, but also IL-8, has been shown to play a pivotal role in the development and progression of atherosclerosis by causing rapid monocyte adherence onto vascular endothelium and subsequent transmigration and activation into the intima (129-133). In this way, the periodontitis-induced enhanced plasma IL-8 and MCP-1 levels and the increased expression in peripheral blood monocytes, may contribute to the progression of atherosclerosis. Periodontitis has been shown to be associated with the formation of more atherosclerosis in the wall of the carotid artery in humans (134). Furthermore, enhanced levels of more common and classical plasma markers of low-grade systemic inflammation, C-reactive protein and fibrinogen, have also proven that periodontitis elicits a systemic inflammatory response (116, 117, 135, 136). However, the enhanced levels of triglycerides and low-density lipoprotein cholesterol in plasma are believed to be the major risk factors for the formation of atherosclerosis, causing endothelial injury or dysfunction and contributing to the subsequent vascular inflammatory response (137-139). It can be concluded that periodontitis might play a role in the progression of atherosclerosis and thereby increase the risk for cardiovascular disease, explaining the associations found between periodontal disease and coronary events (140).

Whereas the expression of the major chemokines IL-8 and MCP-1 by circulating monocytes is primarily meant to recruit and traverse leukocytes to the periodontal lesion, they have also important cellular functions. Especially, the CC chemokine MCP-1 has been shown to be a strong inhibitor of the cytokine IL-12p70 (94), and the levels of MCP-1 consistently correlate in a reversed way with the IL-12p70 levels in LPS-stimulated WBCC from untreated periodontitis patients and controls, as well as in patients after therapy (chapter 4). The cytokine IL-12p70 is a crucial factor in the development of Th1 cells (high producers of IFN-γ), and in the enhancement of innate resistance by inducing IFN-γ production in natural killer cells (69). In addition, due to its pro-inflammatory actions the biologically active IL-12p70 can cause tissue damage and acute toxicity in the peripheral tissues. Therefore, the induced expression of MCP-1 in peripheral blood monocytes from periodontitis patients may
have a counter regulatory effect on the production of IL-12p70, in order to prevent collateral damage when the monocytes arrive in the inflamed periodontal tissues.

Another potent inhibitor of IL-12p70 production in monocytes is the natural chemo-attractant C5a, which is generated by cleavage of the fifth component of the complement system upon activation (94, 141). However, no differences in the plasma C5a levels between periodontitis patients and control subjects without disease, as well as after periodontal therapy, were found (chapter 6). Therefore the decreased monocyte-derived IL-12p70 levels in untreated patients could not be explained by a possible suppressive effect of enhanced circulating C5a levels. Activation of the complement system, or moreover induction of proteins of the acute-phase response, may occur via intruding endogenous or exogenous danger molecules, such as LPS, into the blood stream, in order to mediate the inflammatory response (142, 143). The production of the acute-phase proteins is regulated by early-response cytokines, such as IL-1β, TNF-α and IL-6, which are released by alarmed monocytes or tissue macrophages (144). The acute-phase response naturally extinguishes within a few days due to the short half-lives of the pro-inflammatory mediators, production of anti-inflammatory cytokines, or soluble mediator receptors and receptor antagonists (145). Therefore, no measurable levels of such early-response cytokines were found in almost all periodontitis patients and control subjects without destructive disease (chapter 6). Furthermore, either the acute-phase response disappears or is gradually transformed into a chronic inflammatory disease, e.g. periodontitis, which might be reflected by chronic elevated IL-8 levels in plasma.

Conclusions
Peripheral blood monocytes are highly responsive cells and in addition instructive, and therefore play as APCs a crucial role in connecting the innate and adaptive immunity by orchestrating the modulation of the immune response. Furthermore, as shown for uncommitted immature monocyte-derived DCs that acquire either a Th1-
or Th2-promoting capacity in response to signals delivered by the local environment, peripheral blood monocytes possess also such functional plasticity. This indicates that peripheral blood monocytes may adapt their function to the conditions they are encountered in the blood stream and that changes in this environment, induced either by systemic responses or by danger molecules released from the diseased tissue, results in a different functional profile of the monocytes. In this respect, it might be suggested that monocyte-derived MCP-1 plays an important signaling role, not only by directing the traffic of monocytes to the site of infection, but also by regulating the immune function of these cells, i.e. suppression of IL-12p70 production to reduce the risk of collateral damage to own tissue. It is as yet unknown what causes the functional aberrant phenotype of the peripheral blood monocytes, i.e. enhanced levels of PGE₂ and MCP-1 and down-regulated IL-12p70 levels, as seen in periodontal disease and a variety of other inflammatory diseases. However, there is evidence suggesting that an inverse relationship exists between formation of immune complexes and the generation of Th1 cell responses (146-148). The formation of immune complexes typically accompanies chronic inflammation associated with antigen persistence and the induction of B cell responses, as seen in periodontitis (148). Furthermore, the chronic elevated levels of circulating MCP-1 and IL-8 in periodontitis patients may also have adverse general-health effects, i.e. contributing to the progression of atherosclerosis and therefore increasing the risk for cardiovascular disease. Finally, the persistent enhanced expression of the CC chemokine RANTES in periodontitis patients before as well as after therapy, highly suggests that RANTES may be a true susceptibility factor in the pathogenesis of destructive periodontal disease.

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