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REVIEW

Microbial translocation, residual viremia and immune senescence in the pathogenesis of HIV-1 infection

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Abstract

The pathophysiological mechanisms that underlie the progression of human immunodeficiency virus-1 (HIV-1) disease to full-blown AIDS are not well understood. Findings suggest that, during HIV-1 infection, plasma lipopolysaccharide (LPS) levels, which are used as an indicator of microbial translocation (MT), are elevated throughout the acute and chronic phases of HIV-1 disease. The translocation of bacterial products through the damaged gastrointestinal barrier into the systemic circulation has been described as a driver of immune activation. In contrast, comorbidities that are associated with HIV-1 infection have been attributed to chronic inflammation and immune system dysfunction secondary to MT or low-level HIV-1 replication in plasma and cell reservoirs. Moreover, accelerated aging is significantly associated with chronic inflammation, immune activation, and immune senescence. In this review, we aimed to investigate the role of inflammation as a pivotal marker in the pathogenesis of HIV-1 disease. We will discuss the key features of chronic inflammation and immune activation that are observed during the natural course of the disease and those features that are detected in cART-modified infection. The review will focus on the following aspects of HIV-1 infection: (1) MT; (2) the role of residual viremia; and (3) "immune senescence" or "inflammaging." Many questions remain unanswered about the potential mechanisms that are involved in HIV-1 pathogenesis. Further studies are needed to better investigate the mechanisms that underlie immune activation and their correlation with HIV-1 disease progression.

Key words: Human immunodeficiency virus-1; Combined antiretroviral therapy; Immune activation; Microbial translocation; Residual viremia; Immune senescence

Core tip: The aim of this review was to summarize the most relevant mechanisms in human immunodeficiency virus-1 pathogenesis by focusing on the role of microbial translocation, residual viremia, and immune senescence or "inflammaging" in disease progression to full-blown AIDS. Moreover, the impact of antiretroviral therapy on these mechanisms was investigated.

Fantauzzi A, Falasca F, d'Ettorre G, Cavallari EN, Turriziani O, Vullo V, Mezzaroma I. Microbial translocation, residual viremia and immune senescence in the pathogenesis of HIV-1 infection. *World J Clin Infect Dis* 2013; 3(4): 47-57 Available from: URL: http://www.wjgnet.com/2220-3176/full/v3/i4/47.htm DOI: http://dx.doi.org/10.5495/wjcid.v3.i4.47

INTRODUCTION

Combined antiretroviral therapy (cART) has led to a

lower morbidity and mortality in human immunodeficiency virus type 1 (HIV-1)-infected patients by significantly improving clinical and laboratory parameters. However, the long-term use of cART is associated with adverse side effects that are generally not directly related to HIV-1 infection. These effects include cardiovascular diseases, kidney impairment, osteoporosis, and hepatotoxicities[1]. Moreover, the prolonged survival of patients and the persistence of virus particles in tissue may directly or indirectly contribute to the development of cancers, neurocognitive impairment and a more rapid progression of hepatitis C infection. Chronic inflammation, chronic immune activation, and immune senescence are the pathological hallmarks of HIV-1 infection that lead to these conditions in HIV-1-infected subjects, mainly in subjects with persistently decreased $CD4^+$ T cell counts. Cardiovascular events in HIV-1-infected patients may occur because of the following reasons: (1) these subjects have a higher cardiovascular risk than the general population; (2) the HIV-1 virus can increase the risk of atherosclerosis in patients; and (3) several antiretroviral regimens may influence the atherosclerotic profile of patients due to significant lipidic changes. Therefore, many ischemic cardiovascular events may occur during long-term HIV-1 infection and accelerated atherosclerotic processes may be related either to the infection or to the chronic use of cART[2]. Experimental studies have demonstrated the direct effect of several viral components on the endothelium[3], including the increased expression of adhesion molecules, such as intercellular adhesion molecule and E-selectin; a pro-thrombotic state with increased levels of von Willebrand factor, plasminogen activator inhibitor-1, and tissue plasminogen activator; leukocyte recruitment into the sub-endothelium; and atherosclerotic plaque growth[4,5].

Different factors may contribute to the establishment of immune activation during HIV-1 infection. HIV-1-specific mechanisms and non-specific generalized responses to infection may promote the chronic and aberrant activation of the immune system. An early loss of gut mucosal integrity, the pro-inflammatory cytokine milieu, co-infections, and the subsequent marked destruction of the lymph node architecture are the main factors that contribute to the ongoing activation of the innate and adaptive immune systems. The severe depletion of memory $CD4^+$ T cells, especially cells that express the CCR5 receptor, occurs in the gut mucosa during primary HIV-1 infection and simian immunodeficiency virus (SIV) infection[6].

A massive loss of mucosal T helper 17 (Th17) $CD4^+$ T cells in the SIV-infected rhesus macaque, an animal model of AIDS, has been linked to impaired immune responses in the gut mucosa to an enteric pathogen, which leads to the lack of local control of the pathogen and consequently its translocation[7]. Therefore, both the loss of immune mucosal function and the breakdown of the intestinal barrier may allow the translocation of microbial products into the systemic circulation. Findings suggest that plasma lipopolysaccharide (LPS) levels, which are used as a marker of microbial translocation (MT), are elevated during chronic HIV-1 infection[8]. Regarding cytokine imbalance patterns, higher levels of inflammation markers and coagulation factors, such as high-sensitivity C-reactive protein (h-PCR), D-dimer, and interleukin-6 (IL-6), have been observed in HIV-1-infected patients[9].

Overall, these changes in cytokine and coagulation profiles are associated with an increased risk of cardiovascular diseases, opportunistic conditions, and other mortality causes in subjects with $CD4^+$ T cell counts that are persistently below 500 cells/μL[10,11].

Considering the strong evidence that persistent immune activation is a key cause of HIV-1 disease progression, understanding the mechanisms that drive immune activation during chronic infection is important for developing new strategies that target this process.

CHRONIC IMMUNE ACTIVATION

The current simplified model of HIV-1 pathogenesis integrates the following three main events that occur during the natural or cART-modified course of viral infection: (1) the massive depletion of $CD4^+$ T lymphocytes; (2) paradoxical immune activation; and (3) the exhaustion of immune resources.

These events are briefly analyzed in the following paragraphs and are depicted in Figure 1: (1) During primary infection, HIV-1 can infect a large number of $CD4^+$ T cells, particularly the activated memory T cell subset that expresses the CCR5 ligand. This process is associated with high levels of viral replication[12]. The depletion of $CD4^+$ T cells that is observed in the setting of HIV/SIV (the simian equivalent of HIV) infection is due to the involvement of the central memory $CD4^+$ T cell population. Additionally, this event is based on the establishment of reservoirs of latently infected cells[13,14]. Studies in primates that were infected with SIV and in HIV-1-infected humans have revealed that massive $CD4^+$ T cell depletion occurs in mucosal tissue throughout all of the stages of HIV-1 infection[15]. Plasma HIV-1 viremia (the level of HIV-1 RNA in plasma) increases to peak levels until the adaptive immune response, particularly the onset of HIV-1-specific $CD8^+$ T cells, which generally indicates the end of the acute phase of infection. However, the damage to the immune system is significant: HIV-1 has established a latent reservoir and rooted itself in the host, and extensive viral replication has resulted in the massive depletion of $CD4^+$ T cells, especially in mucosal lymphoid tissue (MALT). Therefore, the compromised integrity of MALT may result in MT from the gut into the systemic circulation[16,17]; (2) HIV-1 infection is associated with chronic immune activation, which appears more pronounced in patients with an advanced cellular immunodeficiency[18,19]. This immune activation is charac-

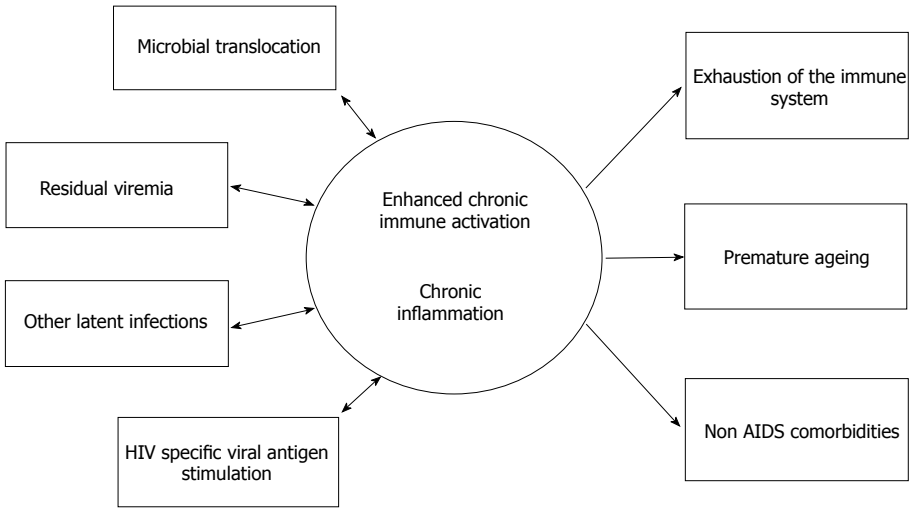

Figure 1 Factors associated with chronic inflammation and immune activation in human immunodeficiency virus-1 disease. HIV: Human immunodeficiency virus-1.

terized by the presence of chronically activated T cells, B cells and monocytes/macrophages; the increased expression of various leukocyte activation markers; the production of pro-inflammatory cytokines; and an increase in cell proliferation[20]. High levels of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), IL-6 and IL-1β, in both plasma and lymph nodes have been observed in the early stages of HIV-1 infection[21,22]. In addition, the secretion of chemokines, such as MIP-1α, MIP-1β and regulated upon activation, normal T cell expressed and presumably secreted (RANTES), is increased in these patients[23,24]. The persistent inflammation status is most likely due to several factors, including the ongoing production of HIV-1; the presence of co-pathogens, such as cytomegalovirus (CMV) or herpes viruses (HSVs); the translocation of LPS across a damaged gut mucosa; the loss of T regulatory lymphocytes and other immunoregulatory cells; and irreversible fibrosis of the thymus and the lymph node infrastructure. CMV causes life-long antigenic stimulation and the subsequent development of an expanded population of well-differentiated, apoptosis-resistant, senescent T cells with limited proliferative potential[25,26]. During HIV-1 infection, the depletion of $CD4^+$ T cells may result in the suboptimal immune control of these persistent viral infectious agents, which permits the reactivation and replication of CMV and Epstein-Barr virus (EBV) infections. Several authors have hypothesized that co-infections with other viruses may contribute to the "accelerated aging" syndrome that is observed in HIV-1 patient populations[27]. Therefore, this state of generalized chronic immune activation is currently considered the hallmark of pathogenic HIV-1 and SIV infections and has a higher independent predictive value of disease progression than viral replication[28]; and (3) During all of the stages of HIV-1 infection, the presence of strong and persistent immune activation is the primary cause of senescence and apoptosis of the immune system and ultimately leads to the exhaustion of immune resources. Immune activation and inflammation result in fibrosis of lymphatic tissue, which damages the lymph node architecture and prevents normal T cell homeostasis[29,30]. Moreover, a vicious cycle is established in which HIV-1 replication promotes immune activation and immune activation promotes HIV-1 replication. Pro-inflammatory cytokines are released and participate in this mechanism. The synergic action of IL-1β, TNF-α and IL-6 can lead to T cell activation. In addition, IL-1βand TNF-α may decrease trans-epithelial resistance in mucosal tissues[31,32]. cART has been considered the best "deactivator" of the immune system in HIV-1-infected patients. cART usually results in a marked reduction in T cell activation and apoptosis[33,34] and a decrease in pro-inflammatory cytokine levels. In addition, antigen-specific stimulation is strongly diminished due to the rapid decline in the number of HIV-1-specific $CD8^+$ T cells[35,36]. cART reduces the depletion of naïve T cells and induces immune recovery. However, even when a decrease in inflammation and the down-regulation of immune activation markers is observed in patients on cART, more inflammatory parameters remain at higher levels than those in healthy individuals and a significant imbalance in the cytokine profiles persists.

MT

The HIV-1-induced disruption of MALT results in the translocation of microbial products across the intestinal mucosa into the peripheral circulation, which produces high levels of plasma LPS and bacterial DNA that persist over time (Table 1). MT is correlated with markers of systemic immune activation[37]. LPS is a component of the cell wall of gram-negative bacteria, and the majority of authors suggest that LPS is a marker of MT throughout chronic HIV-1 infection[38].

Mucosal damage and the dysfunctional phagocytic clearance of microbial products are responsible for MT in the bloodstream. The translocation of bacterial

Table 1 Main dynamics involved in the development of immune dysfunction during human immunodeficiency virus-1 infection

Microbial translocation	Residual viremia	Immune senescence
HIV-1 invasion of the gut mucosa	MT is enhanced in patients presenting residual viremia	High frequency of $CD4^+CD38^+$ and $CD8^+CD38^+$ T cells
Disruption of mucosal integrity and depletion of local Th-17 cells	Stochastic antigen stimulation of long-lived latency infected cells	Accumulation of senescent antigen -experienced memory T
LPS, CpG DNA in blood stream with aspecific and polyclonal immune-activation *via* LPS	Viral replication in anatomical sanctuaries	Inefficient T cell renewal
Pro-inflammatory cytokines secretion (TNF-α; IL-1; IL-6)	Incomplete viral suppression during cART	Fibrosis of lymphopoietic organs cells ($CD28^-CD57^+$)

HIV-1: Human immunodeficiency virus-1; Th-17: T helper-17; LPS: Lipopolysaccharide; CpG: --C--phosphate--G--; TNF-α: Tumor necrosis factor-alpha; IL-1: Interleukin-1; MT: Microbial translocation; cART: Combined antiretroviral therapy.

products results in the profound activation of the innate immune response. LPS, flagellin and CpG DNA, which are toll-like receptor (TLR) ligands, can directly stimulate peripheral macrophages and dendritic cells to produce a range of pro-inflammatory cytokines. Several investigations have demonstrated that LPS is biologically active *in vivo* and its interaction with CD14/TLR-4 on monocyte/macrophages is one of the mechanisms that leads to the secretion of soluble CD14 (sCD14) and pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1. SCD14 is produced by monocyte/macrophages in response to stimulation by LPS. LPS stimulation in vitro has been demonstrated to promote T lymphocyte activation and death[39,40].

The correlation between plasma LPS levels and the frequency of circulating $CD8^+$ T cells with an activated $CD38^+$ HLA-DR+ phenotype suggests that MT may directly or indirectly generate polyclonal T cell activation *via* the production of cytokines and chemokines[41].

Consistent with these observations, subjects with HIV-1 infection and high levels of LPS have an increased risk of disease progression to full-blown AIDS or death, irrespective of their $CD4^+$ T cell counts and HIV-1 RNA levels viral load (VL). Moreover, this marker has been demonstrated to be a strong predictor of mortality, independent of the $CD4^+$ T cell count and the VL[42,43].

The translocation of bacterial products into the systemic circulation through the damaged gastrointestinal barrier has been described as a pivotal driver of immune activation in the course of chronic HIV-1 infection[44-47]. MT is the result of $CD4^+$ T cell depletion in the gut mucosa and increased gut permeability; however, MT has been observed in other diseases, such as idiopathic $CD4^+$ T cell lymphocytopenia[48]. High levels of MT have been observed in many HIV-1-naïve patients. Several studies suggested that cART induces a progressive decrease in the plasma levels of microbial DNA, which tends to stabilize after several weeks of treatment but never normalizes[44]. A reduction in MT and inflammatory markers is broadly associated with a decrease in HIV-1 load. Moreover, recent findings have indicated that the presence of MT is associated with residual viral replication in HIV-1-infected subjects who receive effective cART. Those subjects with higher viral suppression (*i.e.*, VL < 2.5 copies/mL) presented the same LPS levels as HIV-1-uninfected subjects, which suggests that cART may have reverted HIV-1-induced mucosal damage[49]. However, other authors found that MT is strongly associated with higher levels of inflammation markers, independent of HIV-1 VL levels. Despite the findings that cART can reduce MT levels, inflammatory marker levels remain higher than those observed among uninfected subjects[50]. Recent findings in cART-treated subjects revealed that HIV-1 DNA levels in the gut mucosa were strictly correlated with LPS levels and the number of $CD8^+CD38^+$ T cells[51].

Long-term cART is associated with reduced plasma LPS levels and the down-regulation of immune activation markers. However, LPS plasma levels often remain detectable in patients who are successfully treated with cART[44]. This phenomenon may be explained by the ongoing partial repair of the mucosal barrier during cART. The LPS levels in subjects with maximal viral suppression are comparable to those observed in healthy donors. However, the mechanisms of LPS reduction after starting cART are not well understood because these mechanisms do not depend on VL but come into play soon after treatment initiation. However, the lack of an association between reduced MT and increased $CD4^+$ T cells during the first weeks of cART suggests that MT is more influenced by the cellular turnover of latently infected cells than from circulating $CD4^+$ T-cells.

The following questions regarding the role of MT in HIV-1 pathogenesis remain unsolved: (1) During the natural course of HIV-1 disease, does MT contribute to immune system activation or is MT a consequence of immune system activation? (2) Is MT the sole cause of immune activation in HIV-1-infected patients or does residual viremia play a pivotal role? If yes, what is the importance of these two mechanisms? (3) Is gut mucosal damage completely reversible after starting cART? and (4) Moreover, does LPS play a key role in virologically controlled patients with blunted $CD4^+$ T cell gain?

RESIDUAL VIREMIA

The objective of cART is to maintain plasma virologi-

cal suppression below the limits of detection, which are generally less than 50 copies/mL depending on the assay that is used[52]. Several studies have demonstrated that maintaining viral load levels < 50 copies/mL leads to long-term virological success and immunological and clinical benefits in HIV-1-infected subjects. However, the main methods that are used to evaluate HIV-1 RNA load during HIV-1 infection have various detection limits. The polymerase chain reaction (PCR) assay has a detection limit of 400 copies/mL. The ultrasensitive PCR assay has a detection limit of 50 copies/mL, and the real-time PCR assay has a lower limit of detection that ranges from 20-48 copies/mL[53]. The lower limits of detection of the new real-time assays may result in increased measurements of transient and intermittent detectable viral RNA (blips) in patients with virological suppression. There is controversy about the significance and consequences of viral blips. Several authors suggest an association between blips and the development of mutations that confer resistance to cART and an increased risk of virological failure[54-56]. In contrast, other authors did not find any relationship between isolated blips and virological failure[57,58]. Intermittent viremia increases T cell activation and facilitates the extension of HIV-1 infection. Subjects with intermittent viremia present higher levels of total specific $CD8^+$ and $CD4^+$ T cell responses compared with patients who have persistently undetectable HIV-1 RNA levels. These $CD8^+$ and $CD4^+$ T cell responses may block viral replication, thereby reducing the risk of virological failure[59]. The discrepancies in the findings may be due to inconsistencies in the definitions of blips and virological failure and to differences in the testing methods for the detection of HIV-1 RNA levels[60].

Recent studies have used a laboratory-based real-time PCR assay that was capable of detecting single HIV-1 RNA copies/mL. These studies demonstrated that several patients who received cART had persistent low-level viremia that ranged from 1-49 copies/mL. The source and dynamics of persistent viremia in treated patients are currently under investigation. It has been proposed that low-level viremia may be the result of ongoing viral replication in patients, which is caused by incomplete viral suppression during cART[61]. Therefore, several studies have investigated whether intensification with raltegravir, an integrase inhibitor that blocks viral DNA integration into host cell DNA, would further decrease the persistent low-level residual plasma viremia in patients on effective cART[62]. In subjects who were treated during chronic infection, the intensification of cART with raltegravir for 48 wk was associated with a significant decrease in $CD8^+$ T cell activation and a transient increase in episomal HIV-1 DNA, which suggests that raltegravir intensification may positively impact residual HIV-1 replication[63].

The absence of any detectable effects of drug intensification on HIV-1 residual viremia in patients on therapy suggests that viremia is not due to ongoing replication but may arise from different sources. An alternative hypothesis is that the residual amount of HIV-1 RNA may be the result of virus release from long-lived latently infected cells that are activated by stochastic antigen stimulation (Table 1). Several papers have reported that genetically homogeneous viral subpopulations can often be observed in patients on long-term treatment and in the viral population that rebounds during treatment interruptions. These findings further support the concept that persistent low-level viremia arises from long-lived cells rather than ongoing viral replication[64,65].

A further line of investigation has focused on anatomical compartments that may serve as "sanctuary sites", such as the central nervous system and the genital tract, in which HIV-1 replication can occur unhindered by poorly penetrating antiretroviral agents. However, the role of ongoing HIV-1 replication in tissue compartments and cellular reservoirs remains to be defined. Several findings suggest that the reservoir is mainly established and maintained in tissue and that infected cells that are circulating in the blood may not be representative of the much larger population of infected cells in tissue. Sequences of persistent HIV-1 populations in plasma are often not found in peripheral blood resting memory $CD4^+$ T cells[61-65]. Understanding the relationship between residual low-level viremia and the size of the reservoir will help guide future attempts at HIV-1 eradication; however, further prospective studies are required to determine the cause-and-effect relationship between these parameters.

Several studies that have used conventional HIV-1 RNA assays suggest that a chronic inflammation status may persist in patients with undetectable HIV-1 RNA loads[53,66]. The persistency of low-level residual viremia represents a continuous pro-inflammatory stimulus for the immune system, which underlies chronic immune activation and inflammation. Chronic inflammation and immune system dysfunction are important contributors to the increased risk of non-AIDS comorbidities that are often observed in HIV-1 patients, such as cardiovascular events, renal impairment and non-AIDS cancers[41,67]. Moreover, increased levels of inflammation have been associated with an increased risk of progression to AIDS and mortality in HIV-1 patients. In contrast, viremia control is accompanied by a decrease in MT, chronic inflammation and immune system activation parameters[68]. Whether residual low-level viremia plays a key role in increasing the inflammatory status in patients is unclear, and different results have been reported. In the SMART trial, markers of inflammation, coagulation and renal function were elevated in HIV-1 participants and remained elevated even after HIV-1 RNA levels were suppressed with cART[69].

Elite controllers have higher levels of the inflammatory marker C-reactive protein (CRP) than uninfected controls. This finding may be explained by the presence of infected $CD4^+$ T cells that carry replication-competent HIV-1 particles, which suggests that low levels of

ongoing viral replication contribute to the maintenance of HIV-1 reservoirs in the absence of detectable plasma viremia[70]. However, no association has been found between low-level viremia and CRP, fibrinogen and IL-6 levels, which suggests that CRP may not be a reliable marker of inflammation due to ongoing viral replication or viral persistence. In addition, no correlation has been found between immune activation markers and residual viremia[61,66,68]. HIV-1-infected patients with high levels of LPS have an increased risk of progression to AIDS. Plasma LPS levels are correlated with the persistence of HIV-1 in the gut mucosa. Furthermore, HIV-1 DNA levels are correlated with the levels of the activation marker CD38 and $CD8^+$ T cell numbers. Recent studies found that HIV-1-infected patients on cART who had negative HIV-1 RNA plasma levels (< 20 copies/mL) presented less frequently with MT and had lower levels of inflammation markers than patients with low-level viremia (20-200 copies/mL), which suggests that inflammation is induced by MT and not by HIV-1 viremia[50].

These contrasting data indicate that the mechanisms by which residual viremia and chronic inflammation increase the risk of morbidities and mortality in HIV-1-infected subjects on cART are complex, and further studies are needed.

HIV-1 AND IMMUNE SENESCENCE

The association between HIV-1 infection and inflammation is similar to that between advanced age and inflammation, which has been well described. HIV-1 infection shares several similarities with aging, including an increased incidence of cardiovascular diseases, malignancies, infections, chronic viral reactivations, osteoporosis, neurocognitive decline, and frailty[1,71].

Similar to aging, HIV-1 infection is characterized by a general decline in T cell renewal, and an altered capability to regenerate T lymphocytes has been observed in both conditions. Therefore, the naïve T cell pool cannot be efficiently replenished and old, exhausted $CD8^+$ T cell clones and depleted $CD4^+$ T cells cannot be continuously replaced. The double insult of aging and HIV-1 infection impacts the functions of both the hematopoietic stem cell compartment and the thymus and may contribute to many of the changes that are associated with immune senescence, including reduced naïve T cell production, reduced T cell proliferation, and an impaired immune system response to vaccines and infections. The direct infection of the thymic stroma and thymocytes by HIV-1[72-74] and the thymic atrophy that is observed in HIV-1-infected subjects may account for this immune decline, which is similar to age-related thymic involution[75] and may be the result of the suppressive effects of pro-inflammatory cytokines on the thymus[76].

In both aging and HIV-1 infection, the increased expression of the activation marker CD38, which is expressed on the surface of $CD4^+$ and $CD8^+$ T cells, has been observed[77,78]. Moreover, positive correlations have been observed among the proportion of $CD8^+$ T cells that share the HLA-DR+/$CD38^+$ phenotype, the rate of $CD4^+$ T cell decay and the development of opportunistic diseases[79,80]. In addition, persistent T cell activation leads to T cell proliferation and T cell differentiation, which results in the accumulation of senescent, antigen-experienced memory T cells, the reduced expression of CD28 and an increased expression of CD57[81].

The expression of the surface marker CD57 has been correlated with greater resistance to apoptosis in $CD8^+$ T lymphocytes during HIV-1 infection, which facilitates T cell accumulation[82]. CD28 is a co-stimulatory molecule, and the loss of this marker on $CD4^+$ and $CD8^+$ T cells results in reduced B cell function and restricted T cell diversity. A high proportion of $CD8^+$ T cells that express CD57 has been observed in both aging and HIV-1 infection, and this senescent $CD28^-$/$CD57^+$ phenotype is characterized by a reduced capacity to produce IL-2 and a shortened telomere[83,84]. A higher frequency of senescent $CD8^+$ T cells ($CD45RO^+CD57^+CD28^-$) and a lower frequency of naïve $CD4^+$ and $CD8^+$ T cells ($CD45RA^+CD28^+CCR7^+$) were found both in cART-treated patients with undetectable viremia and high $CD4^+$ T cell counts and in older HIV-negative individuals when compared with HIV-1-negative younger controls. The expression of $CD8^+$ T cell activation markers (HLA-DR+$CD38^+$) was higher in HIV-1-infected individuals than in older or younger seronegative individuals[85] (Table 1).

The disproportionate production and accumulation of cytokines, such as TNF-α, IL-1β and IL-6, may lead to several adverse effects. Pro-inflammatory cytokines share a pivotal role in the process of aging and are present at higher concentrations in the blood of the elderly[86,87]. IL-6 is directly associated with the development of age-related disorders, including osteoporosis, cognitive decline and frailty symptoms, whereas increased plasma levels of TNF-α and IL-1β have been observed in the elderly with atherosclerosis[88-90]. In addition, these cytokines may have a role in neurocognitive impairment and neuronal[91-93] pathologies most likely through the induction of large amounts of nitric oxide[94-96], which is conducive to oxidative stress-related damage. This overall process can be referred to as "inflammaging", which is the up-regulation of anti-stress responses and inflammatory cytokines[97]. During the chronic phase of HIV-1 infection, both the accelerated process of immune senescence and inflammaging may contribute to the development of the progressive immunodeficiency.

CONCLUSION

Many questions remain unanswered about the mechanisms that underlie HIV-1 pathogenesis and the role of microbial product translocation, residual viremia and immune senescence in the development of persistent

immune activation and chronic inflammation, which is present at different degrees in all HIV-1-infected subjects. Moreover, despite effective cART-mediated viral suppression, persistent immune activation and inflammation have emerged as a major problem in the current HIV-1 era. Chronic inflammation and persistent immune activation remain abnormally elevated in many HIV-1-infected individuals and can be used to predict disease progression, subsequent mortality and non-AIDS-related morbidities, including cardiovascular diseases.

Different studies have linked inflammatory indexes, cytokine networks, and immune activation markers to clinical outcomes, which validate persistent immune activation as a possible therapeutic target. Other recent investigations have helped to elucidate the role of residual viremia, MT, and immune senescence in driving this persistent inflammatory state. These findings may contribute to the identification of new targets for novel intervention strategies that are aimed at minimizing immune activation and inflammation, such as anti-inflammatory molecules, *i.e.*, corticosteroids, cyclosporine, hydroxychloroquine, aspirin, omega-3 fatty acids, vitamin D and statins. Other interventions, such as IL-2 and IL-7 treatments, may be useful to restore the regenerative capacities of the immune system and to reconstitute the thymic microenvironment and the production of naïve T cells. Experimental strategies that have demonstrated promising anti-aging effects include the use of resveratrol, rapamycin, acetyl-L-carnitine, alpha-lipoic acid, telomerase activators, caloric restriction and stem cell therapy[98].

The effective monitoring of HIV-1-infected patients requires the evaluation of activation biomarkers in clinical practice, which may help guide treatment decisions and may be used to better characterize the infection stage and the risk of disease progression.

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REVIEW

Is there an unrecognised role for *Campylobacter* infections in (chronic) inflammatory diseases?

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Abstract

Campylobacter species are one of the major causes of global bacterial-related diarrheal disease worldwide. The disease is most frequently associated with the ingestion of contaminated meat, raw milk, pets, contaminated water, and the organism may be frequently cultured from the faeces of chicken and other domesticated farm animals. Of the 17 established *Campylobacter* species, the most important pathogens for humans are *Campylobacter jejuni* (*C. jejuni*), *Campylobacter coli* (*C. coli*) and *Campylobacter fetus* (*C. fetus*), which are all associated with diarrheal disease. Further, *C. jejuni* and *C. coli* are also associated with the neuroparalytic diseases Guillain-Barré syndrome and Miller Fischer syndrome, respectively, whereas *C. fetus* is linked with psoriatic arthritis. The discovery of both "molecular mimicry" and translocation-related virulence in the pathogenesis of *C. jejuni*-induced disease, indicates that *Campylobacter*-related gastrointestinal infections may not only generate localized, acute intestinal infection in the human host, but may also be involved in the establishment of chronic inflammatory diseases. Indeed, pathogenicity studies on several *Campylobacter* species now suggest that molecular mimicry and translocation-related virulence is not only related to *C. jejuni*, but may play a role in human disease caused by other *Campylobacter* spp. In this review, the authors provide a review based on the current literature describing the potential links between *Campylobacter* spp. and (chronic) inflammatory diseases, and provide their opinions on the likely role of *Campylobacter* in such diseases.

Key words: *Campylobacter* spp; Infection; Autoimmune diseases; Chronic diseases

Core tip: *Campylobacter* species are able to induce both gastrointestinal and systemic infections in humans and have been linked not only to acute disease, but also to a wide range of (chronic) inflammatory diseases. In this respect, the organism is particularly associated with inflammatory peripheral nerve disease Guillain-Barré syndrome and reactive arthritis. However, the true role of *Campylobacter* in other human inflammatory diseases remains to be determined. This review indicates that the actual role of *Campylobacter* in human inflammatory diseases may be largely underestimated and suggests that further research is necessary in order to accurately determine the importance of *Campylobacter* infection in these diseases.

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INTRODUCTION

Campylobacter species are Gram negative zoonotic hu-

man pathogens that are one of the major causes of global bacterial-related diarrheal disease worldwide, a disease most frequently associated with the ingestion of contaminated animal products such as chicken meat, raw milk, contaminated water, and contaminated farm animals. Currently, of the 17 established *Campylobacter* species the most important associated with human disease is *Campylobacter jejuni* (*C. jejuni*), a leading cause of diarrheal disease worldwide with 400-500 million laboratory confirmed cases each year[1]. Further, this species can be sub-divided into two separate groups based on the presence or absence of sialic acid components attached to carbohydrate residues present on the bacterial outer surface[2]. The transfer of these sialic acid components to the carbohydrate outer surface of *C. jejuni* is mediated by the enzymes sialyltransferase Cst-Ⅱ or Cst-Ⅲ[3], with the presence of sialic acid conferring a highly pathogenic phenotype to the bacterium that has the potential to cause severe colitis[4], as well as paralytic disease. One such paralytic disease is Guillain-Barré syndrome (GBS), a post-infectious life threatening complication often associated with *C. jejuni* infection[5]. In fact, evidence suggests that GBS is facilitated by bacteria-human cross-reactive antibodies, generated *via* a process called "molecular mimicry". Essentially, some sialylated carbohydrate lipooligosaccharide (LOS) structures on the *C. jejuni* outer membrane possess epitopes that appear similar to certain ganglioside epitopes present on human peripheral nerves. This similarity may result in the production of auto-antibodies that target not only the bacterium, but also human nerves, inducing complement-mediated nerve destruction[5]. Further research has also shown that *C. jejuni* strains possessing sialylated LOS structures are significantly more invasive than non-sialylated strains, and are also better able to translocate across the intestinal epithelium[6-8]. In this respect, the authors have previously suggested that infection with *C. jejuni* and other *Campylobacter* spp. may actually be linked with a significant number of undetected bacteremias[9], and that the detection of *Campylobacter* species in current blood culture systems might be underrepresented, not least because these systems are not optimized for the special growth requirements of this bacterial generus[10].

Interestingly, Houliston *et al*[11] established that *Campylobacter* spp. are able to synthesize a much broader range of human mimicking glycolipid/glycoprotein structures in their lipopolysaccharides (LPS) and LOS than previously thought, *i.e*., *Campylobacter* species are equipped with a set of LPS/LOS genes that allow adaptation to their host, possibly allowing the organism to "hide" from recognition by the host immune system. In this hypothesis, LPS or LOS epitopes that mimic host antigens are expressed on the surface of *Campylobacter* bacteria in order to provide protection against the host immune response (*Campylobacter* antigens being recognized as self-antigens and therefore being less likely to be recognized by the host). However, this type of *Campylobacter* LPS and LOS molecular mimicry could potentially be a trigger for the development of as yet unrecognised inflammatory disease states in susceptible hosts. For example, there already exists many publications describing the role of *C. jejuni* in the aetiology of inflammatory diseases such as GBS and Miller Fischer syndrome (MFS), and the reader is referred to[5] for a recent review on this subject.

Worryingly, there are indications that healthy people may actually be (chronic) carriers of *Campylobacter* bacteria, again suggesting that this bacterium is able to adapt itself to the human host and escape immune recognition[12,13]. To confirm these observations, well designed surveillance programs are required in order to identify whether apparently healthy humans can be carriers of *Campylobacter* bacteria[14]. Although the carrier state of *Campylobacter* is not completely clear for humans, it has already been established that various animal species can act as carriers of *Campylobacter* spp. without displaying symptoms[15-19], and animal carriers have been linked to the induction of Campybacteriosis in humans[20-22]. Acute infections with *Campylobacter* species *via* food products, water or animals may also lead to chronic infections in humans[23,24], specifically when patients are suffering from an immunodeficiency[25-28].

In this review, the authors describe and comment on the current literature regarding the potential role of *Campylobacter* spp. in human (chronic) inflammatory diseases. The authors concentrate first on those immunologically-related diseases where a strong association between *Campylobacter* infection and disease has been shown, and then highlight those diseases where an association with *Campylobacter* infection is weaker, but where further research may be warranted. The authors conclude that there is indeed a potential role for *Campylobacter* spp. in the induction of many different types of (chronic) inflammatory diseases, and that this is most likely related to the link between *Campylobacter* infection, inflammation and molecular mimicry.

GBS AND MF SYNDROME

GBS and MF syndromes are (sub)acute inflammatory polyradiculoneuropathies affecting the peripheral nerves of affected patients[5]. GBS and MF patients experience degeneration and demyelination of specific neuronal axons after an episode of gastrointestinal or respiratory infection, with demyelination being triggered by an autoimmune-like response[5]. In GBS patients the muscles in the body become paralysed, whereas in MF patients, only the facial muscles are affected[5].

GBS is also called the Landry-Guillain-Barré-Strohl syndrome and is named after the four scientists that originally discovered and reported on this disease[29]. In 1859 Landry de Thézillat was the first to describe an ascending paralyzing disease in great detail[30], although earlier publications mimicking the disorder of Landry were reported by Auguste François Chomel (1788-1858) in 1828[31] and James Wardrop (1782-1869) in 1834[32]. In 1916 Guillain *et al*[33] reported on an examination of

two soldiers that were suffering from muscular weakness, paresthesias, and muscular pain. In 1927, Draganesco *et al*[34] defined the nomenclature "Guillain-Barré syndrome" to describe this paralyzing disease. In 1982, Rhodes *et al*[35] reported for the first time that the GBS syndrome was associated with *Campylobacter* infection, which was later confirmed by Constant *et al*[36] and Speed *et al*[37]. In all of these studies, it was recognized that diarrhoea often preceded the appearance of Guillain-Barré syndrome, with later bacterial culture and serum diagnostic tests revealing that *C. jejuni* was often the causative agent of the intestinal infection that preceded the onset of the GBS syndrome[38-45].

In 1990, it was suggested for the first time that *C. jejuni* might stimulate the production of antibodies against the myelin sheet of the peripheral nerves of GBS patients[45]. In this same year, Yuki *et al*[46], demonstrated that 2 GBS patients possessed high serum IgG titres against GM1 ganglioside following *C. jejuni* enteritis, a significant finding as these results greatly accelerated the research on *C. jejuni*-induced GBS. Subsequently, multiple research groups confirmed the work by Yuki *et al*[46], linking anti-ganglioside antibodies with *C. jejuni*-induced GBS[47-50]. Eventually, other research groups established that certain *C. jejuni* serotypes (Penner O:4, O:19 and O:41) were more frequently isolated from GBS patients than from enteritis patients, and that anti-GQ1 antibodies were linked to the onset of Miller Fisher syndrome[51-59]. The results of these findings lead to the hypothesis that there exists a form of "molecular mimicry" between *C. jejuni* cell envelope structures and ganglioside structures present on the peripheral nerves of affected patients, suggesting that an immunopathogenic mechanism might be causing damage to the nerves in GBS and MFS disease[60,61]. Indeed, Goodyear *et al*[62], not only showed that monoclonal antibodies raised against the LOS isolated from GBS-inducing *C. jejuni* strains reacted with ganglioside structures on the peripheral nerves, but that they possess the potential to block muscle-nerve interactions. The subsequent finding that *C. jejuni* possesses genes that enable the sialylation of its outer membrane LOS further increased the suspicion that molecular mimicry might form the basis for *C. jejuni*-induced GBS[63-65]. Though mainly associated with *C. jejuni*, research has indicated that molecular mimicry may play roles in human infections associated with several other *Campylobacter* species, particularly with *C. coli*[38,41,66,67].

Based on these findings, there is the potential for an as yet undescribed *Campylobacter*-human autoimmune interactions at the level of molecular mimicry. Further, these interactions could provide the basis for a range of, as yet undefined, chronic and acute inflammatory *Campylobacter*-associated diseases in humans.

INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a term used for a group of chronic inflammatory disorders of the gut that includes two major diseases. These are Crohn's disease (CD) and ulcerative colitis (diseases associated with alteration of the ileum, colon and rectum leading to cramps, fever and bloody diarrhoea). Currently, the association between *C. jejuni* infection and chronic IBDs such as CD is controversial[25,68]. During a four-year study in 1982 in Belgium, 45 patients were found to exhibit signs of an acute infective colitis. Twenty percent of these patients were found to be positive for *C. jejuni,* the bacterium found to be associated with focal colitis, necessitating a differential diagnosis from Crohn's colitis[69]. During the same period, 12 patients were also admitted with an acute attack of IBD due to an intercurrent infection with bacterial pathogens including *C. jejuni*[69]. These data indicate that, in the presence of an acute attack of colitis, an infective etiologic agent should always be sought, and that an attack of chronic idiopathic IBD may be caused by an intercurrent infection. van Spreeuwel *et al*[70] compared 22 patients with *C. jejuni*-induced colitis against 10 healthy controls, 10 ulcerative colitis patients and 10 CD patients, and concluded from immunological analyses that *C. jejuni* colitis can be distinguished from ulcerative colitis and CD patients by IgG containing plasma cells. Also, around the same time, Simson *et al*[71] found that *C. jejuni* was associated with acute relapse and abscess formation in CD. In a Bulgarian study, Boyanova *et al*[24] analyzed the frequency of *Campylobacter* species isolation from patients with acute enterocolitis, IBD, and other chronic intestinal diseases. The authors screened 682 Bulgarian patients and established that *Campylobacter* species were detected in patients with acute enterocolitis (7.8%), chronic enterocolitis (6.2%), CD (6.2%), ulcerative colitis (3.7%), and irritable bowel syndrome (8.3%)[24]. Further, hippurate-positive *C. jejuni* isolates accounted for 62.2% of *Campylobacter* strains. The authors concluded that *Campylobacter* could be one of the causes of chronic intestinal diseases in Bulgaria[24]. Interestingly, Berberian *et al*[72] (1994), showed that the expression of a novel autoantibody defined by the *VH3-15* gene could be detected in both IBD and *C. jejuni* enterocolitis patients. These authors screened 101 individuals with ulcerative colitis, CD or other acute or chronic colitis symptoms. Compared with normal subjects, $BK2^+$ anti-erythrocyte Abs were elevated in most sera from patients with CD and ulcerative colitis (including post-colectomy)[72]. However, $BK2^+$ anti-erythrocyte antibodies were also elevated in 10 of 38 non-IBD patients, all of whom had *C. jejuni* enterocolitis[72]. The findings by Berberian *et al*[72] tended to suggest that a common immunopathogenetic factor, manifested by VH3-15 B cell activation, may be shared between ulcerative colitis, CD, and *C. jejuni* enterocolitis. An indirect effect of *C. jejuni* in the aetiology of IBD was suggested by several different studies of Kalischuk *et al*[73], who established that transcytosis of *C. jejuni* across gut epithelia allowed other commensal gut flora to also cross the intestinal epithelial barrier[68,73,74]. Apparently, the translocation of commensal flora may result in an inflammatory immune response against the commensal gut flora (luminal antigen translocation hypothesis),

a process that is commonly observed in patients suffering from IBD[75,76]. With respect to population studies, Gradel *et al*[77] (2009) compared 13148 people from 2 Danish counties who had been exposed to *Salmonella* and *Campylobacter* gastroenteritis, with 26216 unexposed individuals. After an average follow-up of 7.5 years, the hazard ratio of first-time IBD diagnosis was 2.9 (exposed to unexposed), and was raised for both CD and ulcerative colitis[77]. In those exposed to *Campylobacter* and *Salmonella* only 1.2% of the studied subjects developed IBD. Thus, while the study identified bacterial exposure as a statistically significant factor in IBD the role of *Campylobacter* seems to be only of relative importance. Following the Danish population, but in a different study (1992-2008), Jess *et al*[78] showed that infection with *Campylobacter* species, confirmed by *Campylobacter* isolation from stool samples, significantly increased the risk of developing IBD. However, contradicting this observation, was the finding that culture negative stool samples were also significantly associated with an increased risk for the development of IBD[78]. The final conclusion of the authors was that the increased risk of IBD after infections with intestinal pathogens might be a result of a detection bias, due to increased testing for such pathogens in this patient group[78]. Subsequently, Riddle *et al*[79] further discussed this conclusion stating that due to study limitations and diagnostic bias there could in fact be several different explanations for finding an association between culture positivity or culture negativity in IBD patients.

A more direct link between a *Campylobacter* species (not *jejuni*), CD and IBD was observed more recently. In 2009-2011, Zhang *et al*[80] were able to link the presence of *Campylobacter concisus* (*C. concisus*) to pediatric CD using the techniques of polymerase chain reaction (PCR) bacterial detection and the presence of specific IgG antibody, an observation that has also been associated with adults presenting with IBD[80-82]. Further, in 2011, Kovach *et al*[83] identified actual *C. concisus* proteins that were immunoreactive within patients with CD.

However, not all studies have been successful in linking *C. jejuni* (or any other *Campylobacter* species) with IBDs. In 1984, Blaser *et al*[84] studied 72 CD patients using culture, serology and immunohistochemistry, and concluded that *C. jejuni* was not likely to be an etiological agent of CD or chronic ulcerative colitis. In a Scandinavian study using 95 patients with *Campylobacteriosis,* it was observed that 77%-86% patients harbored a raised antibody titre against an antigen mixture comprising seven *C. jejuni/coli* strains including a PEN 0:6, 7 isolate, which represented the most common serotype in Scandinavia[85]. In this same study, the authors also analyzed the sera of 56 IBD patients and found that none reacted with this *C. jejuni/coli* antigen mixture and concluded that *Campylobacteriosis* was not associated with these chronic diseases[85]. In 1992, a prospective study began by analyzing 64 IBD patients 15 of whom were diagnosed with ulcerative colitis. Stool samples were screened for enteric pathogens, but only a low number of these samples were confirmed to be culture positive. The conclusion of this study was that enteric microorganisms including *C. jejuni* only play a minor or indeed a negligible role in the exacerbation of IBD[86]. Therefore, the link between *C. jejuni* and IBD seems to be weak, but based on current literature, *C. concisus* might by *Campylobacter* species harboring more potential to be a causative agent of IBD.

IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) differs from IBD in the fact that IBS is a collection of gut-related disease signs and symptoms with no known aetiological cause. Though they have several symptoms in common, treatments for IBS and IBD vary greatly. Only a minority of IBS reported disease is associated with a post-infection process (PI-IBS)[87], though one of the commonest causes of PI-IBS appears to be *C. jejuni* infection[88]. The main mechanism for inducing *C. jejuni*-related IBS appearing to be the production of cytolethal distending toxin (CDT) by *C. jejuni*[89]. However, experiments in a rat model have indicated that histopathological changes in the gut during *C. jejuni* infection may be caused by both CDT-producing and non-producing isolates[90]. Therefore the role of *C. jejuni* CDT in IBS remains a point of discussion. One other mechanism involved in the aetiology of IBS is the promotion of inflammation of the gut tissue *via* the generation of a "low grade" immune response (involving autoendocrine cells, CD3, CD4 and CD8 lymphocytes) and gut permeability[91].

Recently, it has been suggested that blockage of the PI3K-γ signalling pathway in *Campylobacter* infection may be a means of reducing severe inflammation facilitated by the innate immune system[92]. Indeed, treatment of *C. jejuni* infection with the antibiotic rifamixin in a rat model of IBS infection stopped the development of long-term altered stool function and form (a phenomenon linked to the overgrowth of the small intestine with *C. jejuni* bacteria, and a characteristic IBS-associated phenotype)[93].

REACTIVE ARTHRITIS (REITER'S SYNDROME)

Reactive arthritis is an inflammation of the joints which develops whilst suffering/recovering from a recent infection. Though other symptoms also usually develop in addition to arthritis, joint inflammation is the main characteristic of this disease. Any site of infection may be associated with reactive arthritis, including the intestine (the site of infection for *C. jejuni*)[94], with symptoms commonly lasting 3-12 mo, though in some cases, the arthritis may persist long-term[94]. Reiter's syndrome a variant of reactive arthritis is established when the following symptoms occur simultaneously; urethritis, arthritis and conjunctivitis[94].

In 1979, a case report was published that linked a *C. jejuni* infection with the induction of reactive arthritis for the first time[95]. In this case report, reactive arthritis de-

veloped two weeks after the subject experienced watery diarrhoea containing blood, and was experiencing anorexia, and severe weight loss[95]. The causative agent of the infection was found to be *C. jejuni*[95]. In a later study, it was established that reactive arthritis was more likely to occur in *C. jejuni* enteritis patients that were positive for histocompatibility antigen HLA-B27[96], and around the same time period different groups more or less confirmed this finding[97-99]. Importantly, HLA-B27-negative arthritis-related *C. jejuni* enteritis cases are nevertheless sporadically reported[97,100-105]. Interestingly, patients presenting with ankylosing spondylitis (a chronic inflammatory disease) overwhelmingly possess HLA-B27 and molecular mimicry with the gut bacterium *Klebsiella pneumoniae* is thought to play a key role in disease development[106,107]. However, research has indicated that there are no signs of *C. jejuni*/*C. coli*-related antibodies in patients with active ankylosing spondylitis[108]. More recently, Mortensen *et al*[4] was able to link a potential virulence factor, namely class A sialylated lipooligosaccharide structures, to a more severe gastro-enteritis phenotype and reactive arthritis, suggesting that sialylated LOS structures, structures that mimic human gangliosides, are also a risk factor in the development of reactive arthritis. Interestingly, the possession/expression of reactive arthritis-related sialylated LOS structures does not appear to be related to any particular *C. jejuni* genotype[109]. For further information, the reader is referred to a systematic review by Pope *et al*[110], which summarizes the link between *Campylobacter* spp. and reactive arthritis.

Up to this point, there has been strong evidence for an association between *Campylobacter* infection and a range of (chronic) inflammatory diseases, GBS, MF, IBD and IBS. However, in the following section, the authors discuss diseases where the link between *Campylobacter* infection and (chronic) inflammatory disease is much weaker. In this respect, the authors would like to see more research in this area, in order to finally confirm or deny any unrecognised association between *Campylobacter* infection and the following diseases.

SYSTEMIC LUPUS ERYTHAMATOSUS

Systemic lupus erythematosus, often abbreviated to SLE or lupus, is a systemic autoimmune disease that can affect any part of the body[111]. As with other autoimmune diseases, the immune system attacks its own cells and tissues, resulting in inflammation and tissue damage[111]. Lupus most often affects the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system[111]. The course of the disease is unpredictable, with periods of illness (flares) alternating with remissions. The disease occurs nine times more often in women than in men, mainly in women in the child-bearing years of 15 to 35, and is also more common in those of non-European descent[111]. Currently, over a 100 articles linking SLE with the GBS have been published, one of the main causative agents of GBS being *C. jejuni*[5]. As early as 1984, Johnson *et al*[112] described a persistent *C. jejuni* infection in a patient with lupus and a deficiency in serum IgA and IgM. The authors showed that the serum of this patient was not able to kill the *C. jejuni* bacterium. In 1998, Gatterbauer *et al*[113] used an ELISA assay to determine the antibody types [IgM, IgG, IgA, and IgG subclass anti-GM1, anti-GQ1b, and anti-asialo-GM1 (anti-GA1)] that were present in patients presenting with neurological or other complicated neurological diseases. Increased anti-GM1 and/or anti-GA1 was found to be more frequent in lupus patients with central nervous system involvement than without[113]. Additionally, in 1999, a case report was published that found antibodies against ganglioside GM1 (indistinguishable from GBS) in the serum of a patient with SLE and a "drop foot", though no antibodies against *C. jejuni* were observed[114]. It has also been reported that an SLE like disease may be triggered in a Balb/c mouse animal model, after immunization of mice with formaldehyde-treated *C. jejuni* and Freud's complete adjuvant[115,116]. However, although *Campylobacter* species may be isolated from lupus patients, it is currently debatable whether *C. jejuni* is the causative agent of lupus disease *per se*, or is simply able to maintain itself in lupus patients due to the immunosuppressive treatment they receive. Particularly interesting are the two articles in which the authors show that they were able to induce an SLE like illness in Balb/c mice using formaldehyde fixed *C. jejuni*[115,116].

CELIAC DISEASE

Celiac disease is an autoimmune disease in which individuals possess antibodies against gluten protein, a protein found in wheat, barley and rye. Sufferers from celiac disease should avoid eating gluten-containing foodstuffs and therefore are subject to dietary restrictions. At least one report has indicated a role for *C. jejuni* in the aetiology of celiac's disease[117]. Additionally, a case report was published in 2010 of a girl suffering from celiac disease that was associated with recurrent Guillain-Barré syndrome (*C. jejuni* being one of the main microorganisms proven to be associated with Guillain-Barré syndrome)[118]. Alaedini *et al*[119] showed increased levels of ganglioside antibodies in celiac disease patients, and suggested that a pre-disposition of celiac patients to bacteria possessing cross-reactive lipopolysaccharides (LPS) such as *C. jejuni* (and *Haemophilus influenzae*), may predispose to the development of anti-ganglioside antibodies (similar to the aetiology of Guillain-Barré syndrome). A similar hypothesis involving tissue atrophy and degeneration of mucosa was also proposed by Sabayan *et al*[120] in 2007.

CARDIOMYOPATHY/MYOCARDITIS

Cardiomyopathy is a measurable deterioration of the function of the heart muscle, usually leading to heart failure. Common symptoms include breathlessness and peripheral oedema (*e.g.*, swelling of the legs). People

with cardiomyopathy are often at risk of dangerous forms of irregular heart beat and sudden cardiac death. The most common form of cardiomyopathy is dilated cardiomyopathy. Myocarditis is an inflammation of the myocardium (heart muscle) and is synonymous with the term inflammatory cardiomyopathy. Interestingly, *C. jejuni* has been linked to cardiac disease in several case reports[121-136]. Also, more severe cases of *C. jejuni* infection may result in heart failure of the patient[123,128,131]. In 2007, Becker *et al*[137] investigated whether the incidence of perimyocarditis is increased following *C. jejuni* infection. Their conclusion after screening 6204 patients for perimyocarditis, and after the patients had experienced a *C. jejuni* infection, was that the incidence rate of myocarditis was 16.1 (95%CI: 2.3-114.4) per 100000 person-year in the *Campylobacter* population compared to 1.6 (95%CI: 0.2-11.4) per 100000 person-year in the control cohort[137]. Although this observation was not found to be statistically significant, the authors did conclude that, based on the rarity of this condition and case reports in the literature linking *Campylobacter* cases with perimyocarditis, it could not be ruled out that a potential association between *Campylobacter* and perimyocarditis might exist[137]. Additional research, indicates that there seems to be a tendency for males to be overrepresented in cardiomyopathy patient groups following *C. jejuni* gastroenteritis symptoms[121-126,138-140], which warrants further investigation. Alzand *et al*[125], suggested that the mechanism by which *Campylobacter* causes myo(peri)carditis could be attributed to direct bacterial invasion of cardiac tissue, bacterial toxins, circulating immune complexes, or cytotoxic T-cells. However, at the moment, the mechanisms leading to cardiac disease after *C. jejuni* infection remain unknown, but support the idea that *C. jejuni* is able to cause systemic infections[9,10].

At this point in the review, the authors present evidence for an association between infection with *Campylobacter* spp. and (chronic) inflammatory diseases, which is based mainly on case reports in the scientific literature.

ACUTE TRANSVERSE MYELITIS

Acute transverse myelitis is a neurological disorder that affects the spinal cord through inflammation, generating for example complications such as axonal demyelination. The disease is associated with an infection or vaccination[141]. In two relatively recent case reports, from 2007 and 2012, acute transverse myelitis was associated with *C. jejuni*-induced gastroenteritis[141,142]. Patients were found to harbor cross-reactive antibodies against the sialylated LOS structures of *C. jejuni*, specifically high titres of anti-GM1 were observed.

GLOMERULONEPHRITIS

Glomerulonephritis is a renal disease that is characterized by inflammation of the glomeruli, or small blood vessels in the kidney[143,144]. It may present with isolated hematuria and/or proteinuria (blood or protein in the urine); or as a nephrotic syndrome, a nephritic syndrome, acute renal failure, or chronic renal failure[144]. Diagnosing the pattern of glomerulonephritis is important because the outcome and treatment differs in different types of glomerular disease[144]. The primary causes of glomerular disease are intrinsic to the kidney[144], but secondary causes of disease may be associated with; certain infections (bacterial, viral or parasitic pathogens), drugs, systemic disorders (SLE, vasculitis), or diabetes[143]. Several case reports have shown a potential link between *C. jejuni* infection and glomerular disease[145-150]. In some reports a *C. jejuni* antigen was identified in the glomeruli suggesting a causal role for this bacterium in the disease process[145,148].

VASCULITIS

Vasculitis is a group of autoimmune diseases in which blood vessels are attacked by the immune system and where inflammation is present[151]. *C. fetus* subsp. *intestinalis* was one the first *Campylobacter* species linked to vasculitis and is seen most often in older, debilitated, or chronically ill men[152]. In case reports, *C. jejuni* has been linked to patients experiencing various forms of vasculitis[114,146,153-158], though whether an actual causal relationship exists between disease and infection is as yet is unknown.

PSORIATIC ARTHRITIS

Psoriatic arthritis is a form of inflammatory arthritis that will develop in up to 30% of people who have the chronic skin condition psoriasis[159]. Psoriatic arthritis is said to be a seronegative spondyloarthropathy and therefore occurs more commonly in patients with tissue type HLA-B27[159]. A strong link between anti-*Campylobacter fetus* antibodies in psoriatic arthritis patients (rheumatoid arthritis, non-arthritic-psoriasis and psoriatic arthritis patients) was observed in the study of Lapadula *et al*[160]. Currently, no further studies on this subject have been reported, and it should be noted that the patient group used in the Lapadula study was small.

CANCER

C. jejuni is phylogenetically closely related to *Helicobacter pylori*, a bacterium established to be a causative agent of gastric cancer[161]. Further, the cytolethal distending toxin of *C. jejuni* may possess DNAse activity and could induce the breakage of double stranded DNA[162], one of the possible steps on the development of cancer. Currently, there is some evidence indicating that *C. jejuni* may possibly be linked to the development of mucosa-associated lymphoid tissue (MALT) lymphoma[163-165]. MALT is a cancer type that originates from B cells in the marginal zone of the MALT, and is also called extranodal marginal zone B cell lymphoma. However, a large

cohort study of Scandinavian patients who had tested positive for *C. jejuni*, and were followed over time (≥ 10 years) showed no increased risk of developing malignancies following an infection by *C. jejuni*[166]. Interestingly, the authors did find a decrease in respiratory cancers following an infection by *C. jejuni*.

CONCLUSION

Campylobacter species are able to induce both gastrointestinal and systemic infections in humans and have been linked not only to acute disease, but also to a wide range of (chronic) inflammatory diseases. In this respect, the organism is particularly associated with the development of neurological diseases such as GBS, MFS, and with reactive arthritis, diseases that are facilitated by the development of cross-reactive antibodies to *Campylobacter* sialylated LOS carbohydrate structures. However, the true role of *Campylobacter*-induced molecular mimicry in other human inflammatory diseases remains to be determined, though this review indicates that the actual role of *Campylobacter* infections in human disease may be largely underestimated. Therefore, further research is required in order to accurately determine the importance of *Campylobacter* infection in a wide range of (chronic) inflammatory diseases of humans.

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MINIREVIEWS

Tuberculosis and hematopoietic stem cell transplant: Review of a difficult and often underestimated problem

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Abstract

Recipients of solid organ transplants (SOT) and stem cell transplants (SCT) constitute a group of patients at risk for tuberculosis (TB) development. The prevalence of active TB in patients undergoing SOT is higher than in patients undergoing SCT, probably due to the shorter period of immunosuppression in the latter. We reviewed the importance of SCT in individuals with hematological malignancies. Most TB cases occur in transplant patients by reactivation of latent infection after immunosuppression, most often within the first year after transplant, leading to graft loss and in some cases, death. Relevant variables to assess the risk of TB infection in a transplant recipient include the donor's and recipient's medical histories, imaging results, microbiology and tuberculin skin test (TST) and interferon-gamma release assays (IGRA). TST is routinely performed in the donor and recipient before transplantation. If TST is > 5 mm in the recipient or > 10 mm in the donor, it is necessary to exclude active TB (pulmonary and renal). Chemoprophylaxis is recommended in TST (+) recipients and in recipients with recent seroconversion, in donors with a history of untreated TB or in contact with an individual with active TB, if radiological images are suspicious and the IGRA is (+). The drug of choice is isoniazid. These topics are herewith reviewed.

Key words: Tuberculosis; Prophylaxis; Transplant; Solid organ transplantation; Hematopoietic stem cell transplantation

Core tip: This review highlights the importance of stem cells transplant (SCT) in individuals with cancer and hematological malignancies. However, the risk of acquiring tuberculosis (TB) in this way, has received little attention, especially in developing countries. SCT candidates should be screened for TB with a careful medical history and chart review to ascertain any history of prior TB exposure, since immunocompromised individuals are at higher risk of latent TB progression to active disease. Finally, we mention the importance of the immune response, particularly in allogeneic stem cell transplants, because infection by intracellular microorganisms such as Mycobacterium TB, could be inhibited by the process named cell reprogramming.

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INTRODUCTION

Stem cell transplant (SCT), also known as pluripotent

hematopoietic cell transplant, was previously referred to as bone marrow transplantation (BMT) since that was the source from which hematopoietic stem cells (HSC) were preferably obtained. This procedure has become an essential therapeutic tool in modern medical practice. As a results of increasing knowledge on SCT, several dogmas that for years hindered development in this area of medicine, have been set aside. It is now established that: (1) the successful collection of HSC does not require the destruction of the receptor's BM; (2) HSC create their own space in the receptor's marrow via graft-*vs*-host effects; (3) several tumors can be eradicated as a result of a graft-*vs* tumor effect; (4) allogeneic SCT (alloSCT) can be conducted on an out-patient basis[1]; (5) allotransplants can be performed in elderly or frail individuals[2]; (6) allogeneic SCT (alloSCT) can be done without red blood cell or platelet transfusions[3]; and (7) in Mexico and in other emerging countries, allotransplant costs can be significantly diminished. These changes have led to increased availability of SCT to a greater number of patients in Mexico and other emerging countries thus offering, in some cases, a real curative option to patients that until recently had no access to this modern therapeutic modality[4]. Transplant recipients constitute a group of patients at risk of developing tuberculosis (TB) and that face great diagnostic and therapeutic dilemmas; the disease's clinical presentation tends to be atypical and the sensitivity of available diagnostic techniques is low. Moreover, anti-TB drugs are highly toxic and frequently interact with anti-cancer agents, rendering disease management difficult. Most TB cases in patients that have undergone transplantation are due to reactivation of latent infections following immunosuppression[5]. We reviewed the relevance of hematopoietic stem cell transplantation (HSCT) in individuals with hematological malignancies and thus at risk of acquiring TB.

EPIDEMIOLOGY

In the past decades, TB prevention programs in developed countries have decreased its incidence; however, in emerging countries it is still high. In Mexico, the incidence of TB in the general population is 14.5/100000 inhabitants, with important regional differences[6]. Mycobacterial infection was uncommon after BMT in the past and, until recently, was considered to be a rare complication, receiving little attention. In North American studies, incidence rates vary between 0.6% and 1%; however, in countries where it is more endemic, its incidence is higher: 1.6% in Spain, 5% in Hong Kong and in Taiwan[7].

A review of BMT patients in large US centers revealed an incidence rate of 0.49%-1%[8] and the scant data available in countries with a high incidence of TB referred frequencies ranging from < 1% to 5.5%[9] and reaching 16% in Pakistan, according to recent reports[10].

However, since the onset of the AIDS epidemic and the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* (MTB), there has been an increasing number of reports of mycobacterial infection in SCT recipients[11]. The lack of a significant response after corticosteroids and the initially predominant involvement of the upper lobes should raise the possibility of pulmonary tuberculosis. A high index of suspicion is important in establishing the diagnosis, and prompt and appropriate treatment will invariably improve the disease's outcome[12]. The reported frequency of MTB infection in solid organ transplant recipients varies from 0.2% to15% (mean, 3.7%), which is 6 to 62 times higher than its frequency in the general population (0.01%-0.045%)[13]. The incidence of TB in the general population is the principal predictor of the increased frequency observed in transplant recipients.

STEM CELL TRANSPLANTATION

SCT is a life-sustaining treatment indicated in some individuals with cancer and hematological malignancies[14]. HSCT refers to the infusion of hematopoietic stem cells obtained from a donor into a patient that has been treated with chemotherapy, usually myeloablative. HSCTs are classified as either allogeneic or autologous, depending on the source of the transplanted hematopoietic progenitor cells. HSCT is defined as any transplantation of blood or marrow-derived hematopoietic stem cells, regardless of the type of transplant (allogeneic or autologous) or cell source (bone marrow, peripheral blood, or placental/umbilical cord blood)[15].

The number of transplants performed in the United States has gradually increased over the last 20 years, particularly in older patients (50 years old). According to the Center for International Blood and Marrow Transplant Research summary report, there were 7012 allogeneic and 9778 autologous transplants performed in 2009[16].

SCT provides an increased chance of survival to patients facing hematological and other potentially life-shortening diseases. These malignancies include acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, aplastic anemia, Hodgkin's and non-Hodgkin's lymphomas, and multiple myeloma. SCT is an expensive treatment and there is wide variation in insurance company coverage, with companies often only paying part of the total expenses. Transplant expenses vary depending on the specifics and type of transplant. A study was conducted between 2000 and 2004 by Saito *et al*[17] at the Dana Farber Cancer Institute/Brigham Women's Hospital, in 376 patients receiving high-dose SCT to estimate costs. The researchers reported median costs of up to $102574 and 36 d of initial hospitalization in a complicated allogeneic SCT.

Hematological disease is frequently accompanied by liver dysfunction. The principal causes of liver injury relating to SCT include: (1) high-dose cytoreductive therapy (chemotherapy and/or radiation) administered prior to transplantation and which may result in veno-occlusive disease (VOD) or nodular regenerative hyperplasia

Table 1 *Mycobacterium Tuberculosis* infections after allogeneic stem cell transplantation

Ref.	Country (period of study)	Patients with TB/No. of HSCT	TB incidence in general population (%)	Site of infection (*n*)
Navari *et al*[20]	United States (1983)	2/682	0.014-0.03	Lung
Kurzrock *et al*[21]	United States (1984)	2/90	0.014-0.03	Lung
Roy *et al*[8]	United States (1974-1994)	11/2241	0.014-0.03	Lung (1), EP (11)
Ip *et al*[22]	Hong Kong (1991-1994)	10/183	5.5	Lung
Aljurf *et al*[23]	Saudi Arabia (1986-1997)	4/641	0.62	Lung, CNS, spine
Budak-Alpdogan *et al*[19]	Turkey (1988-1998)	5/351	1.42	Lung (4), renal (1)
de la Cámara *et al*[11]	Spain (2000)	12/2866	0.41	Lung
Ullah *et al*[24]	Pakistan (2001-2006)	4/154	2.6	Lung (3), EP (1)
George *et al*[25]	India (1986-2001)	9/304	2.3	Lung (2), EP (7)
Lee *et al*[26]	South Korea (1996-2003)	9/295	3.1	Lung (8), EP (1)
Ullah *et al*[27]	Pakistan (2002-2007)	2/37	5.4	Lung
Shima *et al*[28]	Japan (2009)	Case report	-	EP

TB: Tuberculosis; HSCT: Hematopoietic stem cell transplantation; EP: Extrapulmonary; CNS: Central nervous system.

(NRH); (2) liver toxicity due to other drugs used after transplantation; (3) viral and bacterial infections; and (4) acute and chronic graft *vs* host disease (GVHD) in the case of allogeneic transplantation. The differential diagnosis of these complications is guided by knowledge of the timing of their appearance. NRH of the liver is a rare disorder characterized by diffuse micronodular transformation of the hepatic parenchyma, with areas of regenerative activity alternating with areas of atrophy and no fibrous septa between the nodules. Its presentation may be similar to VOD although it is associated with non-cirrhotic portal hypertension and ascites developing after day 100 post-BMT[18].

TB IN ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

In general, TB is rarely seen in alloSCT recipients, but this observation has been challenged in developing countries such as Turkey, where TB infection is more prevalent than in Europe and the United States[19]. In this retrospective study, the incidence of TB infections in 351 alloSCT recipients was reported. The frequency of TB in alloSCT recipients after the allograft (5 of 351) was far greater than that in the general population (35.4 per 100000). Among the 351 patients who underwent alloSCT, 77 subjects that received isoniazid (INH) chemoprophylaxis for 6 mo did not develop post-transplant TB. However, 5 of the remaining 274 patients who received no chemoprophylaxis developed TB a median of 12 mo (range, 10-47 mo) after the allograft (Table 1).

In the bone marrow transplant population, despite severe immune suppression, there is a low incidence of mycobacterial infections[29] that contrasts with the experience reported in other immunosuppressed patients (AIDS and renal transplant recipients). This may be due, at least partly, to the more prolonged duration of immunosuppression in AIDS patients and in recipients of solid organ transplants, when compared with the usual BMT patient[30]. Most patients who develop TB after SCT do not have clearly identified risk factors[31]. Most had normal pre-transplant chest radiographs and no direct history of contact with TB. Although most cases of TB have occurred in alloSCT recipients, 20% have developed in autologous recipients. Despite this low rate[32], diagnostic vigilance must be maintained.

TB among transplant recipients may result from reactivation of quiescent M.tb foci, transmission by the graft or contamination by actively infected individuals. Graft transmission has been documented in renal, lung and hepatic transplants, but accounts for less than 5% of all TB cases in transplant recipients. The risk of TB development in transplant recipients is estimated to be 20 to 50 times higher than in the general population even in developed countries, and mortality rates vary between 20% and 40%. Risk factors include pulmonary images suggesting previous TB infection, immunosuppressive treatment with OKT3 or anti-T cell antibodies, diabetes mellitus, chronic liver disease and coexisting infections[33]. In patients undergoing SCT, associated risk factors include chronic GVHD, allogeneic transplant and total body irradiation[22].

Although accurate diagnosis may be difficult, it is currently possible to hypothesize and/or identify a fungal etiology of pneumonia in SCT recipients; however other pathogens such as *Mycoplasma pneumoniae* or MTB may present clinical and radiological pictures resembling mycosis in SCT patients[34].

PATHOGENESIS OF TB IN HSCT RECIPIENTS

TB is transmitted from person to person by respiratory droplets. Although some people develop active TB disease after infection, almost all TB infections are asymptomatic and remain latent. LTB progresses to active disease in approximately 5%-10% of infected individuals. The rate of progression is much greater in HSCT recipients. The risk of TB in transplant recipients is estimated to be 20 to 50 times higher than in general population even in developed countries, and mortality rates vary from 20% to 40%. Risk factors include pulmonary images suggesting previous TB infection, immunosuppressive treatment with OKT3 or anti-T cell antibodies, Diabetes mellitus, chronic liver disease and coexisting infections (Figure 1).

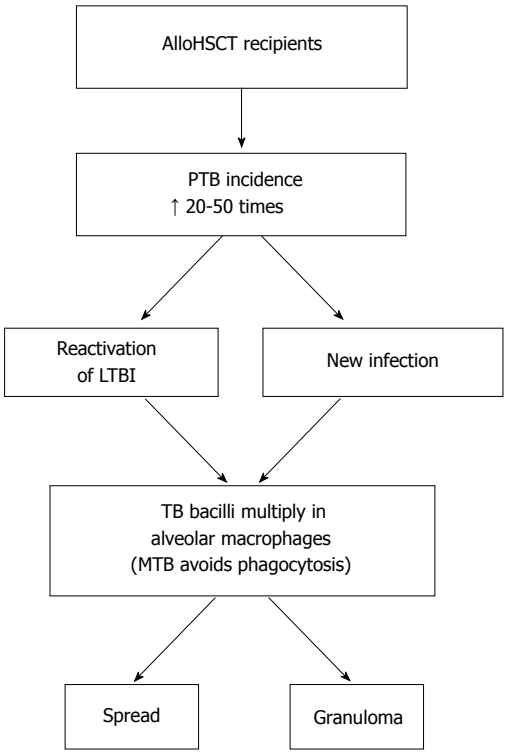

Figure 1 Pathogenesis of tuberculosis in hematopoietic stem cell transplant recipients. AlloHSCT: Allogeneic hematopoietic stem cell transplant; TB: Tuberculosis; PTB: Pulmonary TB; LTBI: Latent TB infection; MTB: *Mycobacterium tuberculosis*.

EVALUATION OF PATIENTS BEFORE SCT

SCT candidates should be screened for TB with a careful medical history and chart review to ascertain any history of prior TB exposure, since immunocompromised individuals have a higher risk of progression of latent TB (LTB) infection to active disease. Also, physicians should apply a tuberculin skin test (TST) using the Mantoux method with five tuberculin units of purified protein derivative or conduct an interferon-gamma release assays (IGRA) The sensitivity and specificity of IGRA testing methods varies according to the used kit type and the study population, and fluctuates between 50% and 100% and 85% and 100% respectively, in different studies (Table 2). Experts disagree on the convenience or benefit of routinely obtaining a TST or IGRA in every transplant candidate. Interpretation of the TST may also be complicated by a history of prior Bacillus Calmette-Guérin (BCG) vaccination, although tuberculin reactivity following BCG tends to wane over time[35]. Any patient with a recent positive TST or IGRA or a history of a positive test and no prior preventive therapy, should be evaluated for active TB. At a minimum, the patient should be asked about symptoms of systemic disease and respiratory symptoms such as cough and shortness of breath, and a chest radiograph should be assessed[36]. Any MTB-mediated disease either in the donor or recipient must be treated until complete microbiological and radiological resolution before considering the possibility of a transplant[37].

Table 2 Salient aspects for diagnosing tuberculosis in a hematopoietic stem cell transplantation recipient

Test	Sensitivity (%)[1]	Specificity (%)[1]	Indicates
TB skin test	72	35	LTBI or active TB
Acid-fast bacillus	50-80	98	Infection
Nucleic acid amplification test	80-98	95-99	Infection
Culture	70-90	98	Active TB
Serology	20-70	47-81	Active TB or infection
Interferon gamma release assays	50-99	85-99	LTBI or active TB
Chest radiography	47-73	76	Probable active TB

[1]These numbers are influenced by the epidemiological situation. LTBI: Latent tuberculosis infection; TB: Tuberculosis.

SCT center personnel should follow guidelines regarding the control of TB in healthcare facilities, including instituting airborne precautions and negative-pressure rooms for patients with suspected or confirmed pulmonary or laryngeal TB. Health care workers should wear N95 respirators, even in isolation rooms, to protect themselves from possible TB transmission from patients with active pulmonary or laryngeal TB, particularly during cough-inducing procedures[38]. SCT candidates and recipients should avoid exposure to persons or environments where there is a substantial risk of respiratory contact with individuals with active TB. It is prudent to advise SCT candidates and recipients that certain occupations (*i.e.*, volunteer work or employment in health care facilities, correctional institutions or homeless shelters) can increase their risk of TB exposure[39].

In SCT patients, a high incidence of TB might be expected due to the complex and severe immunodeficiencies that these patients undergo. Spain has a high incidence of TB (40-45 cases/10^5 inhabitants/year) and a high prevalence of infection (25%-29%) that increases to 56% in individuals > 49 years of age[40], the highest incidence of tuberculosis in Europe after Portugal[41]. It also boasts one of the highest transplant activity in Europe[42]. In a survey of TB after SCT, 20 confirmed cases were found (8 in autologous and 12 in allogeneic transplants) among 8013 patients. TB post-SCT was a late infection (172-324 d), most frequently limited to the lungs (80%) and less frequently, extrapulmonary or disseminated. All SCT patients with TB were symptomatic, fever and cough being the most common symptoms. In allogeneic transplant patients, TB was associated with a high mortality: 25%[11].

INDICATIONS FOR TREATMENT OF LATENT TB INFECTION (LTBI) OR PROPHYLAXIS

Because of the high risk of reactivation or the development of a new infection, prophylaxis should be ad-

Table 3 Clinical manifestations of nontuberculous mycobacterial disease in recipients of hematopoietic stem cell transplant and solid organ transplants

Transplantation type	Mycobacterium species	Types of infection
HSCT	MAC, *M. haemophilum, M. fortuitum, M. Chelonae, M. abscessus*	Catheter-related, pulmonary, cutaneous, disseminated
Kidney	*M. chelonae, M. kansasii, M. haemophilum, M.fortuitum*	Local cutaneous, disseminated, disseminated cutaneous, osteoarticular, pleuro-pulmonary
Heart	*M. kansasii,* MAC, *M. haemophilum, M. scrofulaceum*	Pleuro-pulmonary, disseminated, disseminated cutaneous
Lung	MAC, *M. abscessus, M. haemophilum, M. fortuitum*	Pleuro-pulmonary, local cutaneous, disseminated

HSCT: Hematopoietic stem cell transplant; MAC: *Mycobacterium avium*- intracellulare complex.

ministered to immunocompromised SCT recipients or candidates who: (1) Have been exposed to someone with active, infectious (*i.e.*, sputum-smear positive) pulmonary or laryngeal TB, regardless of the SCT recipient's or candidate's TST or IGRA status; (2) Have a positive TST result-regardless of prior BCG vaccination-without previous treatment and no evidence of active TB disease. A positive TST with a history of BCG vaccination is still considered by the American Thoracic Society as an indication for prophylaxis in patients who "have medical conditions that increase the risk for disease"[36], and which presumably include SCT; and (3) Have a positive IGRA result, without previous treatment and no evidence of active TB.

The report of a high frequency of reactivation of previously treated TB following transplantation, especially in some parts of the world where the endemic TB prevalence is high, suggests that these patients may be at high risk, and therefore, isoniazid (INH) prophylaxis should be considered[26]. LTBI therapy may carry a variable toxicity risk, particularly in the liver and requires strict plasma measurements of immunosuppressive therapy levels. To date, isoniazid is the drug of choice in prophylaxis and has proven effective. The value of prophylaxis in countries with a high rate of LTBI, or in SCT patients from such countries, should be considered at an institutional level.

INH is well tolerated after SCT even with concurrent fluconazole use[43]. Concurrent use of itraconazole is not recommended, and the impact of voriconazole or posaconazole is unknown.

BCG vaccination is contraindicated in SCT candidates. Disseminated BCG infection has been reported among immunocompromised individuals exposed to BCG[44].

Donors who live in or originate from countries where TB is endemic, are at an increased risk of developing TB or LTBI at rates similar to those in their population of origin. There is no known risk in transplanting hematopoietic progenitor cells from an untreated donor with latent or active TB[45].

NONTUBERCULOUS MYCOBACTERIAL INFECTION IN SCT RECIPIENTS

Nontuberculous mycobacteria (NTM) are ubiquitous environmental microorganisms that have generally been considered an uncommon cause of human disease. Before the AIDS epidemic, most cases presented as indolent, cavitating pulmonary infections in patients with other underlying lung diseases, such as chronic obstructive pulmonary disease or previous TB[46]. Mycobacterial infections after transplant have increased in frequency and severity, reflecting both increased exposure and improved diagnostic methods. In countries where TB is endemic, infections due to MTB are more frequent than are infections due to NTM[47].

NTM infections in HSCT recipients have been reported with an incidence ranging between 0.4% and 4.9%[48]. The clinical manifestations of NTM disease in HSCT and solid organ transplant (SOT) recipients are shown in Table 3. The clinical manifestations of disease in HSCT recipients differed from those in SOT recipients. The most common manifestations of NTM disease in HSCT recipients are central venous catheter-related infection, including exit site-related, tunnel-related, and catheter-related blood stream infections. Pulmonary and cutaneous disease is also commonly reported[49].

The most frequently isolated species in HSCT recipients are *mycobacterium avium*-intracellulare complex (MAC) and *M. haemophilum*. Rapidly growing mycobacteria, such as *M. fortuitum* is also common. MAC infection is most often associated with pulmonary or disseminated disease. Rapidly growing isolates have been predominately obtained in catheter-related infections. The presence of *M. haemophilum* has been reported more frequently in SCT recipients than in SOT recipients, usually in association with pulmonary or cutaneous disease but also manifesting as disseminated, osteoarticular, and catheter-related disease.

NTM disease has been reported in recipients of kidney[50], heart[51] and lung transplants[52]. Rapidly growing mycobacteria have been associated with disease in SOT recipients less often than in HSCT recipients.

RELEVANT IMMUNOLOGICAL ASPECTS OF STEM CELLS

Patients receiving transplanted hematopoietic cells undergo a period of immune dysfunction that lasts approximately a year and compromises both cellular and humoral immune mechanisms. This leads to a proclivity to develop infections in the post-transplant period. The

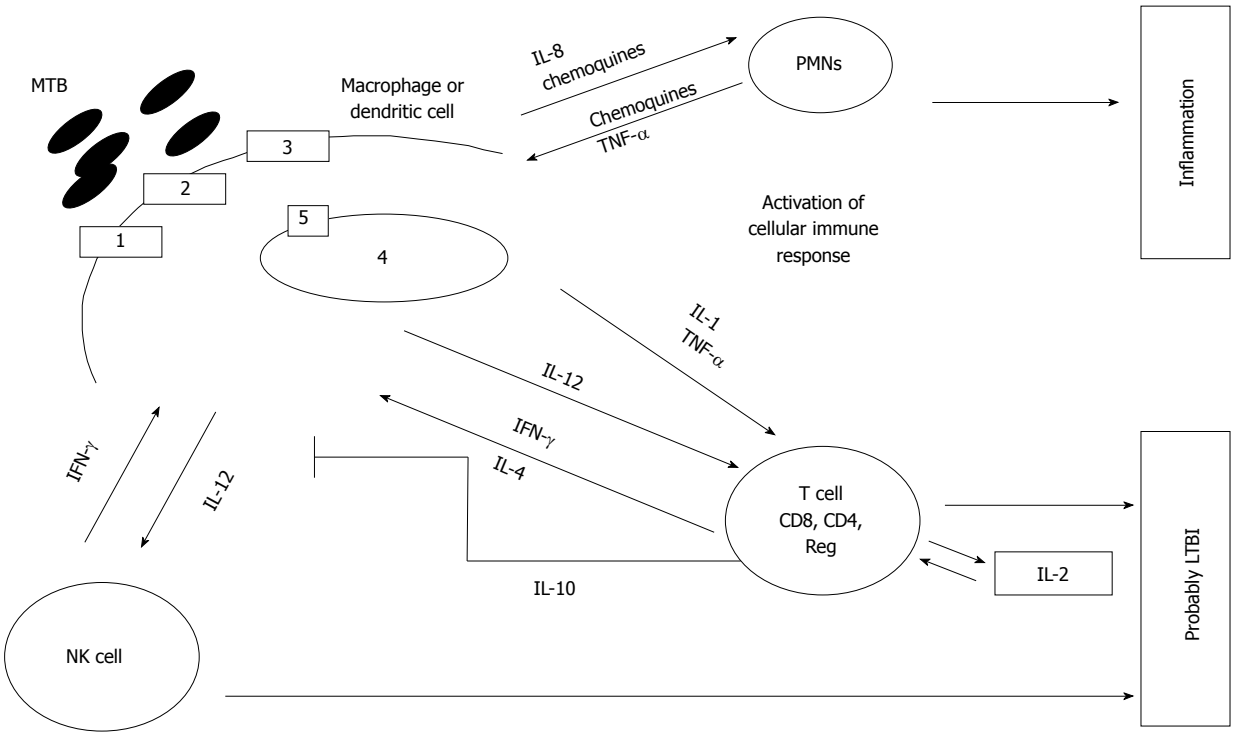

Figure 2 Cellular immune response against tuberculosis. *Mycobacterium tuberculosis* (MTB) recognition by specific receptors and tool like receptors activited signaling pathways that lead to cell activation and cytokine production. The activated macrophages secrete cytokines and chemokines that activate macrophages, T cells and neutrophils, producing inflammation. The T cells and natural killer cells producting gamma interferon with other cytokines that induct activation of macrophages contributing to the elimination of MTB or Latent tuberculosis infection. 1: Complement recepter; 2: Mannose recepter; 3: Scavenger receptor; 4: Phagosome; 5: Toll like receptor. IL: Interleukin; LTBI: Latent tuberculosis infection; NK: Natural killer; TNF-α: Tumor necrosis factor alpha; IFN-γ: Interferon gamma.

conditioning regimen to which patients are subjected destroys normal hematopoiesis and damages different hematological lineages such as neutrophils, monocytes and macrophages as well as mucosal cells, leading to a loss of this barrier's integrity, particularly in the gastrointestinal tract where flora becomes potentially pathogenic.

Recovery of immune function depends on the type of transplant, since in allogeneic transplants the donor's lymphoid cells must learn to recognize an extraneous microenvironment in the absence of a totally functional thymus; this delays immune reconstitution. Another factor affecting the period of immune function recovery is the dosage of cytotoxic therapy in order to allow secondary lymphoid organs to become repopulated. This is applicable in the absence of chronic graft-*vs*-host disease[53].

We must underscore the fact that after hematopoietic stem cell transplant, there is also loss of immune memory in the recipient and the donor's acquired passive immunity unreliably confers long-term immunity[54].

Allogeneic and peripheral blood transplants are associated with a longer immune recovery period than syngeneic and autologous transplants. $CD3^+$ T lymphocytes recover a short time after transplant particularly when more CD34 cells are infused. There is also a prolonged $CD4/CD8$ ratio inversion due to a decrease in $CD4^+$ and an increase in suppressor $CD8^+$ cells[55].

The immunomodulatory capacity of mesenchymal stem cells (MSC), adult multipotent cells, may prevent allogenic rejection by fomenting the avoidance of antigens when interfering with dendritic cell and T lymphocyte function; they thus have a local immunosuppressive effect due to their ability to secrete cytokines. This effect is potentiated when the cells are exposed to an inflammatory milieu or high concentrations of interferon gamma[56,57]. MSC can also inhibit lymphocyte proliferation induced by alloantigens and mitogens such as phytohemagglutinin and concanavalin A or by activation with anti-CD3 and anti-CD28 antibodies. MSC may also inhibit the expression of molecules mediating antigen presentation and in co-culture with peripheral blood mononuclear cells, they increase the proportion of T lymphocyte sub-populations with a regulatory phenotype[58,59].

In hematopoietic stem cell transplants (HSCT), T lymphocyte function remains altered for approximately a year and in allogeneic transplants, the immune repertoire is dominated by donor-derived T cells, particularly effector memory cells[60]. Interleukin-2 (IL-2) production by T Lymphocytes is decreased in response to mitogen stimuli and delayed hypersensitivity reactions are absent and only recover in the absence of Graft-*vs*-Host disease. $CD8^+$ cell cytolytic activity is compromised as underscored by an ineffective response to Epstein-Barr virus[61], a mechanism that may be very similar to that of MTB infection that fundamentally depends on the cellular immune response (Figure 2).

The final phase of cellular immune response recovery hinges on the need for new T lymphocytes from donor pre-thymic precursors in the case of allogeneic transplant.

These cells are processed by the recipient's thymic tissue rendering them tolerant to the allo-environment. There are differences in this lymphocytic "education process" depending on the recipient's age. Children and young patients have a more functional thymus and therefore, an increased recovery in the numbers of T lymphocytes within the first two years after transplantation[62].

In contrast, natural killer cell recovery does not require a functional thymus and develops rapidly within the first weeks after transplant[53]. Although stem cell transplantation is an artificial maneuver, when performed it gears immune mechanisms to take advantage of the stem cells' pluripotent capacity and plasticity; these characteristics are further reflected in organ and tissue regeneration as well as in immune modulation, particularly in immune suppression. Stem cells, particularly MSC, have been shown to inhibit T and B lymphocyte proliferation *in vivo* and *in vitro*, to support the development of regulatory T lymphocytes, to decrease the lytic activity of natural cytotoxic, natural killer and cytotoxic T cells, and to inhibit the risk of infection particularly in the early post-transplant period.

This proclivity to infection particularly in allogeneic stem cell transplants, by intracellular microorganisms such as MTB, could be inhibited by a process named reprogramming in which cells in late differentiation stages reactivate the program of stem cells and recuperate their pluripotentiality.

Tissues can be regenerated by cellular reprogramming and become a treatment strategy for various degenerative disease entities. However, this topic is beyond the scope of this review and is only mentioned because the safety and efficiency of reprogramming methods may represent an alternative, since it imitates the mechanisms used by cells during development; for instance, in cell reprogramming without the introduction of nucleic acids, embryonic fibroblasts have been reprogrammed for the first time with the transduction of the recombinant proteins of transcription factors Oct4, Sox2, Klf-4 and c-myc. However, there are still numerous obstacles to overcome, such as the proteins' short half-lives that require repeated applications and are inherently inefficient[54]. Cellular reprogramming can also be conducted with non-autonomic signals whereby the stem cells destined to a particular organ (multipotent cells) are placed in a similar milieu to that of early embryonic development and are capable of self-reprogramming into a pluripotent state, like embryonic stem cells. Thus, cells from the three embryonic layers (ectoderm, mesoderm, endoderm) can be generated and reflect a state of trans-differentiation[63]. This form of reprogramming is closer to normal cellular ontogenesis mechanisms[64].

CONCLUSION

In summary, transplantation centers should maintain a high level of suspicion of mycobacterial infection during the first 4 mo after transplantation, when mortality due to mycobacterial infections is at its peak. Due to the large numbers of unmatched donors in transplantation programs in countries with high TB prevalences, constant vigilance is required for early detection of mycobacterial infection in SCT recipients. The fact that autologous SCT recipients are immunosuppressed even before transplant, should also be considered.

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MINIREVIEWS

Role of chemokines and cytokines in the neuropathogenesis of African trypanosomiasis

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Abstract

Trypanosoma brucei spp. cause human African trypanosomiasis (HAT) or sleeping sickness in humans and nagana in animals. The early stages of the disease have no specific symptoms; however, the late stage of the disease involves neurological signs of the disease, including disturbance of sleep patterns from which the disease derives the name sleeping sickness. During the late stage of African trypanosomiasis parasites, increased numbers of white blood cells and levels of cytokines and/or chemokines are found in the brain parenchyma and/or cerebrospinal fluid of animal models and HAT patients. In this mini review, contemporary findings on how chemokines and cytokines are thought to play an important role in the central nervous system invasion by the parasites, inflammation and the neuropathology of the disease are discussed. The levels of various cytokines and chemokines, such as interferon-gamma (IFN-γ), interleukin-1 beta (IL-1β), IL-6, IL-10, tumor necrosis factor-alpha (TNF-α), C-C motif chemokine 2 (CCL2), CCL3, C-X-C motif chemokine 8 (CXCL8, IL-8) and CXCL10, in the cerebrospinal fluid (CSF) of HAT patients correlate with the severity or stage of the disease. Thus, these molecules are possible candidates for differentiating between early and late stage HAT. The role of cytokines and chemokines in parasite invasion of the central nervous system is also being elucidated. IFN-γ, TNF-α and CXCL-10 are some of the cytokines and chemokines now known to facilitate parasite penetration of the brain parenchyma. Interestingly, they also constitute some of the candidate molecules with potential to differentiate between stage 1 and 2 of HAT. The increased levels of cytokines, such as IL-1β, IL-6, IFN-γ and TNF-α, as well as prostaglandins, during African trypanosomiasis might contribute to the neurological dysfunctions that occur during HAT.

Key words: African trypanosomiasis; Chemokine; Cytokine; Central nervous system; Brain parenchyma; Cerebrospinal fluid; Neuroinvasion; Neuroinflammation; Neurological disturbances

Core tip: Human African trypanosomiasis (HAT) or sleeping sickness, caused by Trypanosoma brucei spp., is staged into an early hemolymphatic stage and a late meningoencephalitic stage. During the late stage parasites, increased numbers/levels of white blood cells, cytokines and/or chemokines are found in the cerebrospinal fluid of patients. In this mini review, contemporary findings on how chemokines and cytokines, such as interferon-gamma (IFN-γ), TNF-α, C-X-C motif chemokine 8 (CXCL8) and CXCL10, are thought to play an important role in the central nervous system invasion by the parasites, inflammation and the neuropathology of the disease and what might be candidates to differentiate between early and late stage HAT are discussed.

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INTRODUCTION

Three morphologically identical subspecies of the hemoflagellate protozoan parasite *Trypanosoma brucei* (*T. b.*), *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense*, cause African trypanosomiasis, the latter two species are human infective. The disease is endemic to sub-Saharan Africa and is transmitted through a bite of a tsetse fly (Glossina sp.) during a blood-meal. *T. b. gambiense*, which is found in foci in large areas of West and Central Africa, causes a chronic form of human African trypanosomiasis (HAT) that lasts for several months to years. On the other hand, *T. b. rhodesiense*, with a much more limited distribution, is found in East and Southeast Africa and causes an acute form of the disease that lasts for several weeks to months[1,2].

Clinically, HAT is divided in to two stages: an early hemolymphatic stage (stage 1) and a late encephalitic stage (stage 2)[1,3,4]. However, the demarcations between these two stages of the disease are not clear, more so for disease caused by *T. b. rhodesiense* where there is rapid transition from stage 1 to stage 2[3]. In the early stage of HAT, a chancre might develop at the site of inoculation, followed by involvement of blood and lymphatic systems, which presents with general signs and symptoms of infection, chronic intermittent fever, headache, lymphadenopathy, splenomegaly and pruritus. In the late stage of the disease, there are signs of nervous system involvement, which present as sleep disorders, *i.e.*, dysregulation of the circadian rhythm of the sleep-wake cycle and a fragmentation of the sleeping pattern, neurological symptoms, including confusion, tremor, fasciculations, general motor weakness, hemiparesis, akinesia or dyskinesia, sensory disturbances with diffuse hyperpathia, abnormal movements and speech disorders, and psychiatric symptoms. If untreated, the disease will lead to coma and death in most cases. The patients die in a state of cachexia and also because of opportunistic infections[4]. Clinical symptoms of HAT are of a non-specific nature; thus, its diagnosis is confirmed by finding trypanosomes in the blood and lymph nodes or in the cerebrospinal fluid (CSF) using microscopy, the latter during the late stage of HAT. The serological test card agglutination trypanosomiasis test is used to screen for *T. b. gambiense* infections. The World Health Organization criteria for diagnosing stage 2 HAT is the finding of trypanosomes and/or a white blood cell (WBC) count of > 5/μL in the CSF[1,4].

Differentiating between the two stages of the disease is imperative before treatment can be begun[1] because of the differences between the drugs used to treat early and late stages of HAT in terms of ability to cross the blood-brain barrier (BBB) and toxicity. The drugs which are used to treat the late stage of the disease, melarsoprol, eflornithine and the nifurtimox-eflornithine combination treatment, permeate the BBB better but are more toxic than the drugs used to treat the early stage of the disease, suramin and pentamidine[3,4].

In this mini review, the role of chemokines and cytokines in the invasion of the central nervous system (CNS) by the parasite and the ensuing inflammation and neuropathology which makes the disease intractable and fatal in most cases will be discussed. Cytokines are a large group of immunoregulatory molecules. They play an important role in the control and pathogenesis of infectious diseases. Chemokines are involved in recruitment and retention of immune cells during inflammation and infection.

Table 1 Cytokines and chemokines with increased expression in the brain parenchyma of rodents and cerebrospinal fluid of human patients, more during late than early stage African trypanosomiasis

Site	Cytokine/chemokine	Ref.
Chemokines		
Rodent brain parenchyma	CCL2[1], CCL4, CCL5, CCL7, CCL9, CCL12, CCL19, CCL28 CXCL1, CXCL5, CXCL9, CXCL10[1], CXCL12, CXCL13[1], CXCL14, CXCL16,	[5,19]
HAT patient CSF	CCL2[1], CCL3, CXCL8 (IL-8), CXCL10[1], CXCL13[1]	[5,7,8,20-25]
Cytokines		
Rodent brain parenchyma	IFN-γ[1], IL-1α, IL-1β[1], IL-6, IL-10[1], TGF-β, TNF-α[1]	[10,19,26-28,30]
HAT patient CSF	IFN-γ[1], IL-1β[1], IL-6[1], IL-10[1], TNF-α[1]	[5,7,8,20,22,32-34]

[1]Expressed in both late stage rodent brains and HAT patients' CSF. CCL: C-C motif chemokine; CSF: Cerebrospinal fluid; CXCL: C-X-C motif chemokine; HAT: Human African trypanosomiasis; IFN: Interferon; IL: Interleukin; TGF: Transforming growth factor; TNF: Tumor necrosis factor.

CHEMOKINE AND CYTOKINE EXPRESSION IN THE CNS

Trypanosome infection results in activation of the immune system and induction of expression of various cytokines and chemokines in both HAT patients and animal models of the disease[5-11]. However, eventually the infection results in immunosuppression[12-14]. The cytokines and chemokines that are induced, both in the periphery and the CNS, play an important role in the control of the parasites but they also contribute to the inflammation and immunosuppression which occurs during the disease[6-11,15-17].

Increased expression of chemokines in the CNS has been observed during African trypanosomiasis. The expression of the chemokines C-X-C motif chemokine (CXCL) 1, CXCL2 (macrophage inflammatory protein-2, MIP-2), CXCL5, CXCL9, CXCL10, CXCL12, CXCL13, CXCL14, CXCL16, C-C motif chemokine (CCL) 2 (MCP-1), CCL3 (MIP-1alpha), CCL4, CCL5 (RANTES), CCL7, CCL9, CCL12 and CCL28 was found to be up-regulated in the brains of rodents infected with *T. b. brucei*[5,9,18,19]. Some of these chemokines are expressed at higher levels during late than early stage African trypanosomiasis (Table 1). CXCL9 and CXCL10 were the most highly up-regulated cytokines in the brain at later stages when parasites had invaded the CNS, compared to early stages of the disease before CNS invasion. The

Table 2 Cytokines and chemokines involved in Trypanosoma brucei spp. neuroinvasion

Cytokine/Chemokine	Trypanosome levels in the brain parenchyma of transgenic mice compared to WT mice	Ref.
Chemokines		
CXCL10	CXCl10$^{-/-}$ and CXCR3$^{-/-}$ mice had less trypanosomes in the brain parenchyma compared with WT mice.	[5]
Cytokines		
IFN-α/β	IFN-α/βR$^{-/-}$ mice had slightly less trypanosomes in the brain parenchyma compared with WT mice.	[15]
IFN-γ	IFN-γ$^{-/-}$ and IFN-γR$^{-/-}$ had less trypanosomes in the brain parenchyma compared with WT mice. Trypanosomes accumulated in the perivascular compartment, confined between the endothelial and the parenchymal basement membranes, in certain areas of the brains of both transgenic mice	[10]
IL-12	IL-12P40$^{-/-}$ mice had less trypanosomes in the brain parenchyma compared with WT mice.	[10]
TNF-α	TNFR1$^{-/-}$ mice had less trypanosomes in the brain parenchyma compared with WT mice.	[15]

CXCL: C-X-C motif chemokine; IFN: Interferon; IL: Interleukin; TNF: Tumor necrosis factor; WT: Wild type.

increased expression of both chemokines was found to be dependent on interferon (IFN)-γ[5]. CXCL10 was found to be predominantly up-regulated in parenchymal astrocytes of hypothalamic regions, optic chiasm and optic tracts at later stages of the disease[5]. Of these chemokines, CCL2, CCL3, CXCL8 (IL-8), CXCL10 and CXCL13 have been found to be increased in the CSF of patients with late stage HAT infected with either *T. b. gambiense* or *T. b. rhodesiense* more than non-infected control patients or patients with early stage HAT (Table 1)[5,7,8,20-25].

Several studies have reported the increased expression of cytokines in the CNS during trypanosome infection. The cytokines IFN-α/β, IFN-γ, interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-10, IL-13, transforming growth factor (TGF)-β and tumor necrosis factor (TNF)-α were found increased in the brains of rodents infected with *T. b. brucei*[10,18,19,26-30]. Some of these cytokines are also expressed at higher levels during late than early stage African trypanosomiasis (Table 1). It has been suggested that astrocytes might be the source of some of these cytokines since the levels of these cytokines were found to correlate with astrocyte activation[19]. Lymphocytes are the major source of IFN-γ in the brains of *T. b. brucei* infected mice[10]. *T. b. brucei* CpG-DNA stimulates macrophages to increase the production of IL-12 and TNF-α[31]; thus, macrophages and possibly microglia might be some of the major producers of these cytokines in the brain during *T. b. brucei* infections. Of these cytokines, IFN-γ, IL-1β, IL-6, IL-10 and TNF-α have been found to be increased in the CSF of patients with late stage HAT infected with either *T. b. gambiense* or *T. b. rhodesiense* more than non-infected controls or patients with early stage HAT (Table 1)[5,7,8,20,22,32-34]. On the other hand, the level of TGF-β was decreased in the CSF of patients with late stage HAT infected with *T. b. rhodesiense* compared to patients with early stage HAT but was higher than a non-infected control, where it was not detected in the latter[34].

CYTOKINES, CHEMOKINES AND TRYPANOSOME BRAIN INVASION

Taking into consideration that the expression of various chemokines and cytokines in the CNS correlate with presence of trypanosomes in the brains of animal models of the disease and CSF of HAT patients, there is a possibility these molecules play a role in the recruitment, mobility and retention, and also in the control of the levels, of the parasites in the CNS. The role which some of these molecules play in trypanosome invasion of the brain have been studied using transgenic animal models (Table 2)[35].

Of these molecules, the role of IFN-γ in trypanosome invasion of the brain was the first to be studied using transgenic mice[10]. Mice deficient of IFN-γ or its receptor had higher parasites in the blood but had less parasites and lymphocytes in the brain parenchyma compared to wild type (WT) mice. In these transgenic mice, the parasites accumulated in the perivascular space between the endothelial and parenchymal basement membranes of the post-capillary venules[10], suggesting that IFN-γ or factors induced by it are important for parasite crossing of the parenchymal basement membrane. The source of the IFN-γ was most likely lymphocytes since the levels of IFN-γ did not increase in recombination activating gene deficient mice (lacking mature B and T lymphocytes) and parasite penetration into the brain parenchyma was reduced in these mice. Mice deficient of IL-12 have reduced IFN-γ levels[36] and also have less parasites penetrating the brain parenchyma[10].

IFN-γ induces the production of the chemokine CXCL10, also known as IFN-γ-induced protein 10 (IP-10). Mice deficient of IFN-γ have reduced expression of CXCL10 compared to WT mice during trypanosome infection[5]. Transgenic mice lacking CXCL10 or its receptor CXCR3 also showed reduced parasites penetrating the brain parenchyma, although they had similar parasites in the blood compared to WT mice[5]. CXCL10 deficient mice did not have accumulation of parasites in the perivascular space, suggesting that other IFN-γ-induced molecules instead of CXCL10 play a role in IFN-γ dependent passage of parasites across the parenchymal basement membrane.

The role of TNF-α in trypanosome invasion of the brain was also studied using transgenic mice[15]. Mice deficient of TNF-α receptor 1 had higher parasites in the blood but had less numbers of both parasites and T lymphocytes in the brain parenchyma compared to WT mice[15]. *T. b. brucei* infected mice deficient of TNFR1

Table 3 Selected cytokines and associated neurological and neuroendocrine features of African trypanosomiasis

Cytokine	Possible neurological and neuroendocrine features associated with	Ref.
IFN-γ	Sleep pattern disruptions, hyperalgesia/hyperesthesia and pain	[41,60]
IL1β	Hyperalgesia/ hyperesthesia and pain, neurodegeneration	[28,29,61]
IL-6	Hypopituitarism and endocrine dysfunctions, sleep pattern disruptions, hyperalgesia/ hyperesthesia and pain	[38]
TNF-α	Hypopituitarism and endocrine dysfunctions, sleep pattern disruptions, hyperalgesia/hyperesthesia and pain, neurodegeneration	[28,29,38,41]

IFN: Interferon; IL: Interleukin; TNF: Tumor necrosis factor.

had less adhesion molecules, vascular cell adhesion protein 1 and intercellular adhesion molecule 1 compared to WT mice, suggesting that the induction of adhesion molecules through TNF-α signalling might play a role in the TNF-α facilitated parasite and T cell invasion of the brain parenchyma. Mice deficient of IFN-α/βR had reduced numbers of T lymphocytes and parasites in the brain parenchyma compared to WT mice, but the magnitude was not as pronounced as mice deficient of IFN-γ, TNF-α or CXCL-10 signalling[15]. Mice lacking the receptors of two other cytokines, IL-1R and IL-18R, had similar parasites in the blood, as well as parasites and T lymphocytes in the brain parenchyma, as WT mice[15], suggesting that these cytokines do not play a significant role in parasite penetration in to the CNS.

CYTOKINES, CHEMOKINES AND NEUROPATHOLOGY DURING AFRICAN TRYPANOSOMIASIS

Chemokines such as CXCL10 play a role in the attraction, mobility and/or retention of inflammatory cells into the CNS during African trypanosomiasis[5] and thus contribute to the neuroinflammation and morbidity seen in the late stage of the disease. High levels of CCL2, CCL3, CXCL8 and CXCL10 in the CSF were found to be associated with the severity of the disease and neurological signs which are characteristic of late stage HAT[5,7].

High levels of cytokines, such as IL-1β, IL-6, IFN-γ and TNF-α, in the plasma or CSF have also been found to be associated with the severity of the disease and neurological signs which are characteristic of late stage HAT[7,8,22,36,37]. However, as well as neuroinflammation, the role of cytokines in causing brain dysfunctions which result in neuroendocrine dysfunctions, neurological symptoms and/or sleep disorders (Table 3) have also been studied. In HAT patients, high plasma concentrations of IL-6 and TNF-α have been found to correlate with hypopituitarism and endocrine dysfunctions[38]. Endocrine dysfunctions result in some of the signs and symptoms of HAT, such as impotence, amenorrhea, infertility and lethargy. Chronically elevated concentrations of IL-6 and/or TNF-α during HAT might have a direct inhibitory effect on the hypothalamus-pituitary-thyroid or adrenal axis, resulting in reduced thyroid hormone and cortisol secretion[38]. TNF-α inhibitors have been shown to restore the hypothalamic-pituitary-adrenal axis in other chronic inflammatory diseases, such rheumatoid arthritis[39].

IL-6, IFN-γ and TNF-α can alter synaptic functions and are implicated in causing sleep pattern disruptions[40-45]. IFN-γ alters clock gene expression and circadian rhythms in the suprachiasmatic nucleus (SCN)[41,46]. The SCN is essential for the generation and maintenance of daily rhythms in physiology and behavior[47-49]. IL-1β, TNF-α and IFN-γ also affect hypothalamic and brainstem neurons which are involved in sleep-wakefulness regulation[41,50,51]. Apart from HAT, IL-6 and/or TNF-α are elevated in other disorders associated with excessive daytime sleepiness, such as sleep apnea, narcolepsy and idiopathic hypersomnia[43,44,52].

Cytokines and chemokines can sensitize and stimulate nociceptors in the periphery and/or synaptic targets in the CNS, which can result in neuropathic pain[53]. Administration or up-regulation of IL-1β, IL-6, IFN-γ and TNF-α can induce neuropathic pain in rodents[40,54-59]. It has been suggested that IL-1 and IFN-γ might be implicated in the thermal hyperalgesia observed in *T. b. brucei infected* rats[60,61]. Hyperesthesia is one of the clinical features reported in HAT patients[3,4]. Thus, these cytokines, together with other inflammatory molecules, most likely contribute to the hyperalgesia/hyperesthesia and pain observed in HAT.

In rats infected with *T. b. brucei*, apoptosis of some cells and degeneration of some nerve fibres, although modest, in the brain have been found to be spatially associated with mRNA expression of the cytokines IL-1β and TNF-α[29]. Intraventricular infusion of an antagonist of TNF-α, but not IL-1, was found to reduce trypanosome-induced neurodegeneration[28]. Infusion of antagonists of both cytokines further reduced the trypanosome-induced neurodegeneration; thus implying that TNF-α is a principle mediator of trypanosome-induced neurodegeneration and its effects are augmented by IL-1[28].

CONCLUSION

The expression of cytokines and chemokines in the brain and/or CSF is increased in animal models of African trypanosomiasis and HAT patients and the levels of these molecules correlate with the severity or stage of the disease. The high levels of chemokines and cytokines in the brain and CSF during late compared to early stage African trypanosomiasis are most likely due to the invasion of the CNS by trypanosomes and/or WBCs in the late stage, resulting in neuroinflammation. Thus, these molecules are possible candidates for differentiating between early and late stage HAT. In the future,

clinicians could utilize this knowledge to treat patients with high levels of these molecules in the CSF as late stage patients; thus, possibly reducing the occurrence of relapses in late stage HAT patients who might have been wrongfully diagnosed as early stage and treated as such using the current staging criteria. Recently, extensive research has been undertaken to evaluate the suitability of these molecules as stage biomarkers and also as markers for treatment outcome in HAT patients[5,7,8,20-25,32-34]. The role of cytokines and chemokines in parasite invasion of the CNS is also being elucidated. IFN-γ, TNF-α and CXCL-10 are some of the cytokines and chemokines now known to have a facilitative role in parasite penetration of the brain parenchyma. Interestingly, they also constitute some of the molecules with potential to differentiate between stage 1 and stage 2 of HAT[5,20,22]. Moreover, neopterin, a stable product produced by IFN-γ activated immune cells, has been suggested to have potential to differentiate between these two stages of HAT[24]. The increased levels of cytokines, such as IL-1β, IL-6, IFN-γ and TNF-α, during African trypanosomiasis contribute to the neurological dysfunctions that occur during HAT. Thus, studying cytokines and chemokines during African trypanosomiasis not only aids in understanding the neurobiology of the disease, but also provides candidate diagnostic markers and possible therapeutic targets to reduce the neurological sequelae in surviving patients.

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CASE REPORT

Primary lymphocutaneous nocardiosis associated with gardening: A case series

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Abstract

Most cases of nocardiosis are seen in immunocompromised patients. Primary lymphocutaneous is a relatively uncommon presentation of this disease that may also occurs in normal hosts. Diagnosing this infection requires a high index of suspicion since cultures can take several days to exhibit growth. The microbiology laboratory must therefore be notified about cases in which this pathogen is suspected. We report four cases of primary lymphocutaneous norcardiosis. Of particular interest is the association of three of these cases with gardening.

Key words: Nocardia; Nocardiosis; Lymphocutaneous; Brasiliensis; Asteroides; Gardening

Core tip: Nocardiosis is an infection most often seen in immunocompromised individuals. In particular, primary cutaneous disease rarely occurs in normal hosts. We present a case series of patients that developed this infection after gardening. As nocardial infections are frequently mistaken for routine pyogenic processes and as routine cultures are rarely kept long enough to show growth, this condition should be considered in patients with such a history and cultures should be incubated for several weeks.

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INTRODUCTION

Nocardia species are ubiquitous soil-dwelling Gram-positive branching beaded bacteria that are responsible for a wide spectrum of diseases in both normal and immunocompromised patients[1,2]. Nocardia infections of the pulmonary system and disease dissemination, including secondary cutaneous involvement, are well documented in immunosuppressed patients[3,4]. We report four cases that demonstrate the classic features of primary lymphocutaneous nocardia infection. Three of these infections were associated with gardening.

CASE REPORT

Case 1

A 49-year-old Hispanic man with a history of diabetes mellitus presented with swelling and redness in the left elbow after squeezing a furuncle on his left hand 2 d prior. He had no fever and did not recall any injury or trauma to the hand. On physical exam, he had a furuncle with visible pus on the dorsum of the left ring finger. He also exhibited erythema and edema up to the left elbow with streaks of lymphangitis. He had a normal white blood cell count (WBC) count of 7.0 K/μL and an elevated high-sensitivity C-reactive protein (hs-CRP) of 6.6 mg/L.

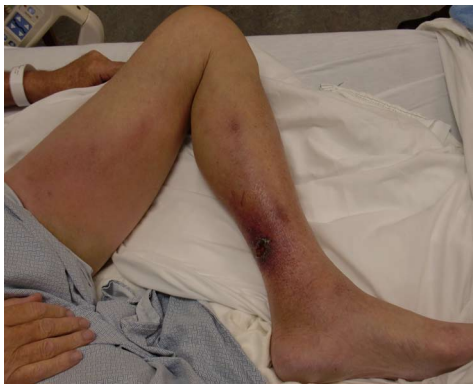

Figure 1 ***Nocardia brasiliensis*** **cutaneous abscess with lymphangitis.**

He was admitted and treated with intravenous clindamycin for 2 d. The lesion was incised and drained and the purulent fluid was sent for cultures. He was discharged on oral cephalexin and trimethoprim-sulfamethoxazole for suspected methicillin-resistant Staphylococcus aureus. The Gram stain revealed the presence of Gram-positive beaded rods 3 d after admission. *Nocardia brasiliensis* was found by sequence identification 25 d after the initial admission. Treatment with oral trimethoprim-sulfamethoxazole was continued for 6 mo.

Case 2

A 59-year-old Hispanic man with a history of esophageal cancer with metastases to the lung and hypertension presented to his primary care physician with left calf redness, swelling and pain. The symptoms started after manipulating a furuncle on his left calf. Two days prior to this, he had been working in his garden and had knelt down in soil. He had not received any chemotherapy in the last 2 years. He was started on oral cephalexin but after 2 d, he developed rigors, diaphoresis and fever. He therefore returned to the emergency room and was admitted. On physical exam, he was afebrile and had a 1 cm ulcer with purulent discharge on the posterior aspect of his left calf. The surrounding area was erythematous and tender but not fluctuant or indurated. He had an area of erythema on the medial aspect of the left thigh with tender left inguinal lymphadenopathy. The largest lymph node was about 1 cm in diameter. The patient exhibited lymphangitis along the medial aspect of the lower extremity (Figure 1). He had a normal WBC count of 8.2 K/μL, elevated CRP of 31.2 mg/L, alkaline phosphatase of 341 U/L, AST of 41 U/L and total bilirubin of 2.2 mg/dL. The wound was incised and drained and the fluid sent for cultures. He was started on vancomycin and piperacillin/tazobactam. The Gram stain showed Gram-positive beaded rods 3 d after admission. He was discharged on oral trimethoprim-sulfamethoxazole. Final cultures were positive for *Nocardia brasiliensis* by sequence identification 37 d after admission. Treatment with oral trimethoprim-sulfamethoxazole was continued for a total of 6 mo with complete resolution of symptoms.

Case 3

A 78-year-old Caucasian man with a history of prostate cancer and coronary artery disease presented to his primary care physician with a right knee lesion that had started as a small furuncle one week prior. He had been gardening and kneeling in the soil recently. Due to concerns for septic arthritis, an aspiration of the knee was attempted but no fluid was recovered. He was started on an outpatient regimen with oral doxycycline. After 2 d, he developed a new erythema over his right thigh. He therefore returned to the emergency room and was admitted to the hospital. On physical exam, he was afebrile and his right knee was erythematous. He had a suppurative lesion in the subpatellar region and there were multiple indurated and erythematous areas in a linear pattern from the patella to just below the femoral ligament. There was no inguinal lymphadenopathy. He had a normal WBC count of 8.6 K/μL and an elevated CRP of 56 mg/L. The area was incised and drained and the fluid sent for cultures. He was started on intravenous vancomycin. He was discharged after 2 d on oral trimethoprim-sulfamethoxazole. Fungal cultures were positive for partial acid-fast thin-branched filaments 6 d after admission. The organism was identified as *Nocardia brasiliensis* 39 d after admission. Trimethoprim-sulfamethoxazole was stopped because of a rising creatinine level and he was switched to amoxicillin/clavulanate and minocycline. The treatment was continued for a total of 6 mo with complete resolution of symptoms.

Case 4

A 62-year-old Caucasian man living in the Bahamas presented to his primary care physician with a right knee lesion associated with pain, redness, swelling and warmth for the last 3 d. About a week prior to developing these symptoms, he had noticed a small scratch in the same area. This wound had not been covered while gardening and he had been kneeling in soil on his bare knees. He had a past medical history of ulcerative colitis being treated with infliximab. As his symptoms worsened, he decided to seek care at our facility. On physical exam, he was afebrile, had a 2 cm × 3 cm × 1 cm abscess on the right knee, right inguinal lymphadenitis and signs of lymphangitis along the medial thigh. He had a normal WBC count of 7.0 K/μL and an elevated CRP of 24.5 mg/L. His alkaline phosphatase was 69 U/L, aspartate aminotransferase (AST) 67 U/L, and alanine aminotransferase 45 U/L. He was treated with intravenous ceftriaxone and his infliximab therapy was put on hold. Gram positive beaded rods were seen on the Gram stain 3 d after admission. The organism was identified as *Nocardia brasiliensis* 10 d later. He was then switched to oral trimethoprim-sulfamethoxazole. After 6 mo of therapy, his infliximab therapy was resumed. Given the need for treatment with his immunomodulator, he was continued on prophylactic trimethoprim-sulfamethoxazole indefinitely.

DISCUSSION

The Nocardia genus belongs to the order Actinomyceta-

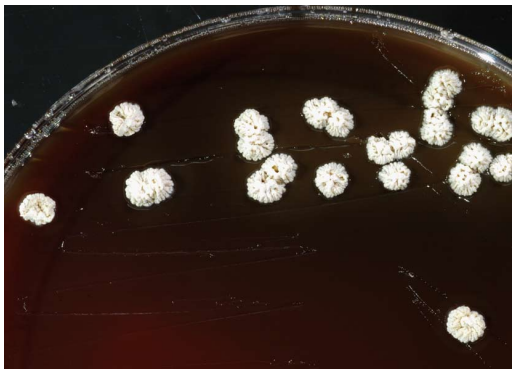

Figure 2 *Nocardia brasiliensis* white, rugged colonies on blood agar.

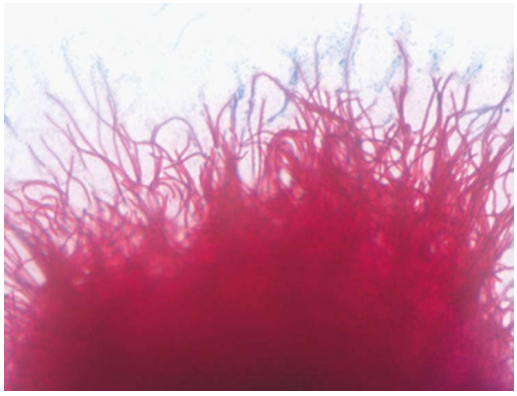

Figure 3 Modified acid fast stain of *Nocardia brasiliensis* demonstrating filamentous growth.

les, a group of gram-positive, aerobic, branching, beaded, partially acid-fast bacteria[5]. They are ubiquitous in nature and can be found in soil, air, and water[1,6]. *Nocardia asteroides* is the most common species associated with human disease[7]. There may be geographic variation in Nocardia species distribution with more case of *Nocardia brasiliensis* observed in the Southern United States[4].

The exact incidence of nocardiosis is difficult to estimate but approximately 1000 cases occur annually in the United States[8,9]. Nocardia infections of the pulmonary system and disease dissemination, including secondary cutaneous involvement, are well documented in immunosuppressed patients[1,2]. Primary cutaneous nocardiosis accounts for up to 5% of all cases of nocardiosis and is more often associated with *Nocardia brasiliensis*[2,3,10,11].

Primary cutaneous nocardiosis results from direct inoculation of the organism into the skin as a result of minor trauma, thorn puncture or insect bite[5,10,11]. Primary cutaneous nocardiosis may cause ulceration, pyoderma, cellulitis, nodules or subcutaneous abscesses. Patients commonly present with pain, swelling, erythema and warmth[2,3,6]. It can be clinically indistinguishable from other bacterial infections such as the ones caused by *Staphylococcus aureus* and group A streptococci.

Lymphocutaneous nocardiosis occurs when a primary nocardial skin infection involves and spreads within the lymphatic system. The clinical picture of lymphocutaneous nocardiosis could look similar to infections caused by *Sporothrix schenckii* or atypical mycobacterial infection[7,10]. However, in nocardia infections, the presentation is usually more acute.

Nocardia are relatively slow-growing organisms that can be grown on standard blood agar but prefer enriched media such as Lowenstein Jensen or Sabouraraud-dextrose agar. They typically appear as white, yellow, or orange rugous colonies (Figure 2). On microscopy, nocardia appears as aerial filaments that break up into small bead-like spores (Figure 3). Routine cultures usually require 5-21 d to exhibit growth, which may lead to underdiagnosis if the organism is not seen on Gram staining since most routine cultures are discarded after 3 d of incubation[6].

The standard treatment for nocardiosis is trimethoprim-sulfamethoxazole. Surgical debridement of purulent lesions may be needed to optimize therapy. While no definitive length of treatment has been established, the patient should be treated for at least 6 mo to prevent relapse. Longer treatment courses should be considered in immunosuppressed patients[2,6,8,10,12].

Our case series is of particular interest because three of the four patients had had recent exposure to soil while gardening. In addition, two of them recalled having a small wound on their knee prior to kneeling on soil. We believe that the patients inoculated themselves with the organism at that time. We therefore suggest that a careful history should be taken with specific questions about soil exposure in patients presenting with symptoms similar to those described. In addition, we suggest that a modified acid-fast stain be performed and that cultures be kept for 21 d in such cases.

Nocardiosis is an infection most often seen in immunocompromised individuals. Infections most commonly affect the lungs and are more often associated with *Nocardia asteroides*. Primary cutaneous disease rarely occurs in normal hosts and is typically associated with significant soil contact. In these cases, the main pathogen is *Nocardia brasiliensis*. Nocardial infections are frequently mistaken for routine pyogenic processes, as routine cultures are rarely kept long enough to show growth. Sulfa-containing regimens are often effective in treating nocardia infections and treatment should be continued for at least 6 mo.

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

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16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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