Summaries

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Leishmaniasis, resulting from infection with the protozoan parasite Leishmania, consists of a wide spectrum of clinical manifestations, from healing cutaneous lesions to fatal visceral infections. A particularly severe form of cutaneous leishmaniasis, termed mucosal leishmaniasis, exhibits decreased IL-10 levels and an exaggerated inflammatory response that perpetuates the disease. Using a mouse model of leishmaniasis, we investigated what cytokines contribute to increased pathology when IL-10-mediated regulation is absent. Leishmania major infected C57BL/6 mice lacking IL-10 regulation developed larger lesions than controls, but fewer parasites. Both IFN-γ and IL-17 levels were substantially elevated in mice lacking the capacity to respond to IL-10. IFN-γ promoted an increased infiltration of monocytes, while IL-17 contributed to an increase in neutrophils. Surprisingly, however, we found that IFN-γ did not contribute to increased pathology, but instead regulated the IL-17 response. Thus, blocking IFN-γ led to a significant increase in IL-17, neutrophils and disease. Similarly, the production of IL-17 by cells from leishmaniasis patients was also regulated by IL-10 and IFN-γ. Additional studies found that the IL-1 receptor was required for both the IL-17 response and increased pathology. Therefore, we propose that regulating IL-17, possibly by downregulating IL-1β, may be a useful approach for controlling immunopathology in leishmaniasis.

Cutaneous leishmaniasis is caused by the protozoan parasite Leishmania where the severity of the disease is a function of both parasite replication and the immune response. These obligate intracellular parasites infect phagocytes, such as macrophages, and are controlled when macrophages become activated by IFN-γ. Thus, a Th1 response is a required component in controlling the disease. However, the immune response itself can contribute to the pathology associated with this infection. The most extreme example of this is in mucosal or mucocutaneous leishmaniasis, although it is important to point out that even in localized cutaneous leishmaniasis the immune response is largely responsible for the lesions that develop [1]–[6]. Thus, it is the inflammatory response, rather than uncontrolled parasite growth, that often perpetuates the disease. For this reason, regulatory mechanisms that dampen the immune response are critical for controlling the disease. Indeed, cells from patients with mucosal leishmaniasis produce less IL-10 than those with localized cutaneous disease, and cells within the mucosal lesions exhibit a reduced expression of the IL-10 receptor (IL-10R) [2], [7]–[9]. These observations suggest that the lack of IL-10 or responsiveness to IL-10 may be an important contributing factor in the immunopathology observed in this disease. A better understanding of the pathogenesis of mucosal disease is important, since drug therapy is often not successful in these patients [10]–[13]. It is believed that high levels of IFN-γ and TNF-α contribute to the disease, which might provide targets for immunotherapy [14], [15]. More recently, increased levels of IL-17 have been identified in patients with cutaneous and mucosal leishmaniasis, suggesting that IL-17 may also play a pro-inflammatory role in this disease and could be a target for immunotherapy [16], [17]. Moreover, BALB/c mice lacking IL-17 develop significantly smaller lesions than control mice [18]. On the other hand, IL-17 has been associated with protection against human visceral leishmaniasis and was required in a vaccine model [19], [20]. Thus, the role of IL-17 in leishmaniasis remains poorly understood, and further studies are required to determine if blocking IL-17 will be therapeutic in severe cases of cutaneous leishmaniasis. C57BL/6 mice infected with L. major develop lesions similar to those seen in patients with localized cutaneous leishmaniasis, and after 10 to 12 weeks the lesions resolve. Healing is associated with the development of a Th1 response, leading to increased levels of IFN-γ, activation of macrophages, and subsequent killing of the parasites by nitric oxide. These protective responses are held in check by the production of IL-10, since C57BL/6 mice lacking IL-10 produce higher levels of IFN-γ and are better able to control the parasites [21], [22]. IL-10 also regulates Th2 responses in leishmaniasis. Thus, BALB/c mice develop an uncontrolled disease that occurs concomitantly with a dominant Th2 response, while BALB/c Il10−/− mice resolve their infections [23]. L. major infected Il27r−/− mice develop more severe pathology than wild-type mice, and also exhibit decreased IL-10 levels concomitant with a Th2 response that may also contribute to the disease [24], [25]. Thus, IL-10 has previously been shown to be important in regulating the immune response to leishmaniasis, but a murine model that mimics the severe pathology seen when an exaggerated Th1 type immune response develops in leishmaniasis remains unexplored. To develop a model to study Th1 associated pathology in leishmaniasis, we infected C57BL/6 mice in the ear with L. major, while simultaneously inhibiting IL-10 effects with anti-IL-10 receptor mAb. This treatment created mice that were IL-10 signaling deficient (IL10SD). IL10SD mice, as well as L. major infected Il10−/− mice, developed significantly larger lesions than the controls, but had fewer parasites within the lesions. We found that IL-10 was primarily produced by CD4+Foxp3+ and CD4+Foxp3− cells. As expected, there was a significant increase in the production of IFN-γ in IL10SD mice early after infection, which mediated both increased recruitment of monocytes as well as iNOS production. We also found a significant increase in IL-17 production and neutrophil accumulation in the lesions of IL10SD mice. Therefore, we next addressed the question of whether IFN-γ or IL-17 (or both) was required for the development of immunopathology in the absence of IL-10 signaling. Instead of decreasing pathology, IFN-γ neutralization in IL10SD mice resulted in increased pathology as assessed by lesion size, with a substantial increase in IL-17 production and neutrophil infiltration. In contrast, IL-17 neutralization in IL10SD mice abrogated the increased pathology seen in IL10SD mice. Similarly, we found that IL-17 production by cells from leishmaniasis patients were regulated by IL-10 and IFN-γ Finally, since IL-1β was significantly elevated in IL10SD mice and is critical for IL-17 production in many systems [26]–[29], we assessed the course of infection in Il1r1−/− mice that were treated with anti-IL-10R mAb. We found that Il1r1−/− mice failed to develop severe pathology when treated with anti-IL10R mAb, and correspondingly showed reduced IL-17 levels and the lack of neutrophil recruitment. Taken together, these results indicate that IL-17 can mediate extensive pathology in leishmaniasis if not regulated by IL-10, and that in the absence of IL-10, IFN-γ plays a critical role in regulating IL-17 production.

Since the lack of IL-10 or IL-10 receptor expression has been linked with the increased pathology seen in mucosal patients, we investigated whether neutralization of IL-10 responses would lead to a more severe disease. C57BL/6 mice were infected with L. major and treated for 4 weeks with either anti-IL-10 receptor mAb (IL10SD mice), or isotype control mAb. IL10SD mice developed significantly larger lesions than control mice (Figure 1A). While the lesions were larger, the parasite burden was about 1000-fold less than control mice by the fifth week of infection (Figure 1B). Similar results were obtained in Il10−/− mice (Figure 1C, D). The lesions in IL10SD mice were not only larger in size, but also substantially more ulcerated (Figure 1E). In fact, as the lesions increased in size some of the IL10SD mice lost substantial ear tissue after 4 weeks of infection, which impacted measuring lesions, such that there was an apparent decrease in lesion size of IL10SD mice. Of note, severe pathology continued to be apparent weeks after anti-IL-10R administration was discontinued, suggesting that IL-10 produced early after infection is critical in the subsequent lesion development. Histologically, the lesions were associated with a leukocytic infiltration in the dermal layer with a predominance of mononuclear and polymorphonuclear cells. In particular, the lesions in IL-10SD mice exhibited microabscesses in the dermis and marked epidermal thickening compromising the stratum corneum, spinous and basal layers (Figure 1F). Several cell types can produce IL-10 following infection with L. major [23], [30], [31]. We reasoned that the critical IL-10 associated with the induction of increased pathology was produced relatively early after infection. Therefore, we utilized IL-10 transcriptional reporter mice to define the cells within the lesions that were making IL-10 at one week of infection. We observed a substantial increase in IL-10 expression by CD4+ T cells following infection. In addition, CD8 T cells infiltrating the lesions produced IL-10, and there was a slight increase in IL-10 production by CD11b+ and NK1. 1+ cells, but no changes in IL-10 production by TCRγδ T cells or B cells (CD19+) (Figure 2A). In naïve mice IL-10 was primarily produced by CD4+ Foxp3+ cells (Figure 2B). However, following infection the IL-10 came from both CD4+Foxp3− and CD4+ Foxp3+ T cells, and about 50% of the CD4+Foxp3− T cells producing IL-10 also produced IFN-γ (Figure 2B, C). In order to determine if blocking IL-10R signaling altered the ability of these cells to make IL-10, we compared IL-10 production by CD4+ T cells (both Foxp3+ and Foxp3−) in IL-10SD and control mice. The absolute number of IL-10 producing of CD4+Foxp3− and CD4+ Foxp3+ T cells did not differ between controls and IL10SD mice. However, we found a modest, although significant, decrease in the percentage of IL-10 producing regulatory T cells (Tregs) when IL-10R was blocked. A more dramatic effect was observed in CD4+Foxp3− T cells, where the treatment reduced the frequency of IL-10 producing Foxp3− cells to that observed in naïve mice (Figure 2D). These results indicate that the ability of Th1 cells to make IL-10 is partially dependent upon IL-10 itself. Whether the IL-10 initiating IL-10 production by CD4+ T cells is produced in an autocrine manner, or from other cells, such as macrophages that are known to produce IL-10 following interactions with Leishmania parasites [23], is unknown. IL-10 suppresses immune responses, and thus not surprisingly we found increased T cell responses in IL10SD mice. We harvested the draining lymph nodes from control and treated mice, and stimulated these cells with leishmanial antigen to assess what differences we might observe in cytokine production. There was a significant increase of IFN-γ production in response to leishmanial antigen stimulation in cells from treated mice (Figure 3A). In contrast, there was a decrease in IL-4 production, which most likely is due to an increase in IFN-γ (and IL-12) in IL10SD mice. In addition to the development of a stronger Th1 response, we also found that T cells from treated mice produced more IL-17 (Figure 3A). Little to no IL-17 was produced by cells from L. major infected (isotype) controls, suggesting that not only did IL-10 neutralization increase cytokine responses that were already present (e. g. IFN-γ), but also uncovered responses that were low or non-existent. We next examined the production of IFN-γ and IL-17 within the lesions at 1 and 5 weeks after infection. As early as 1 week after infection, there was a significant increase in the percentage and number of Th1 cells present within the lesions compared with naïve or control (isotype treated and infected) mice (Figure 3B). Nevertheless, by 5 weeks, IFN-γ responses were similar in treated and untreated mice. There is a high frequency of IL-17 producing cells in naïve mice that is probably dependent upon the skin commensal microbiota [32]. Interestingly, the frequency of IL-17 producing CD4 T cells within the ear decreased in infected mice at 1 week, which is presumably due to the recruitment of CD4 T cells that did not make IL-17. However, the number and frequency of IL-17 producing cells in IL10SD mice was increased compared with the infected controls. In addition, there was an increase in the mean fluorescence intensity in the Th17 cells (Figure 3C). The CD4+ Th17 cells that developed in IL10SD mice failed to co-produce either IFN-γ or IL-10 (Figure 3D, E) as reported in other systems [33], [34]\_ENREF\_11. There was also a slight increase in IL-17 production by CD8 and γδ T cells in IL10SD mice compared with controls (data not shown). Thus, in IL10SD mice there is an increase in both Th1 and Th17 cells compared with infected controls. In order to better understand the development of disease in the anti-IL-10R mAb treated mice, we characterized the early cellular infiltrate within the lesions. Myeloid populations were identified as CD11b+ cells, and further classified based on their expression of additional markers (Ly6C, Ly6G, CD11b and MHCII). We found a significant increase in the frequency and absolute number of CD11b+ cells by 1 week of infection (Figure 4A). Monocytes (Ly6C+) and neutrophils (Ly6G+) were recruited to leishmanial lesions soon after infection, and at 1 week we saw an increase in both of these cell populations in the infected control mice. However, in IL-10SD mice we found significantly larger numbers of both monocytes and neutrophils. Thus, while approximately 40% of the CD11b+Ly6G− cells were monocytes in infected control mice, this increased to more than 70% of the CD11b+ Ly6G− cells in IL-10SD mice (Figure 4A). Similarly, an increased frequency of neutrophils contributed to the early increase of CD11b+ cells within the lesions of treated mice (Figure 4A). By 5 weeks neutrophils dominated the lesions in IL10SD mice (Figure S1). Administration of anti-IL-10R mAb also promoted the activation status of CD11b+ cells. Thus, a large percentage of the CD11b+ cells recovered from the site during the first week of infection of IL10SD mice were significantly more activated than infected control mice, as measured by iNOS and MHC class II expression on CD11b+Ly6C+ cells (Figure 4B). This increase in recruitment and activation of monocytes was completely T cell dependent, since it failed to occur in anti-IL-10R mAb treated Rag−/− mice (Figure S2). To determine if IFN-γ controlled this early monocyte response, we treated mice with anti- IFN-γ during the first week of infection, and assessed monocyte recruitment and activation. We found a significant decrease in the recruitment of monocytes to the site of infection (Figure 5A). Additionally, MHC class II and iNOS expression was reduced in both infected C57BL/6 and IL10SD (Figure 5A). Strikingly, we found that neutralization of IFN-γ led to a significant increase in the lesion size by 1 week of infection. This was not due to a change in the parasite burden at this early time point (Figure 5B), but rather was associated with a substantial increase in the recruitment of neutrophils to the site of infection, such that in IFN-γ neutralized IL10SD mice 75% of the CD11b+ cells in lesions were neutrophils (Figure 5C). This increase in neutrophils corresponded with an increase in IL-17 production (Figure 5D). These results are consistent with previous observations that IFN-γ regulates IL-17 responses [35]–[37]. More importantly, this result suggests that instead of IFN-γ promoting increased pathology, IFN-γ may be critical for downregulating IL-17 responses. Thus, in the absence of IFN-γ an uncontrolled IL-17 response leads to a dramatic increase in neutrophils and subsequent pathology. To determine if IL-10-mediated regulation of IL-17, as describe by others [38]–[40], could also be observed in patients with leishmaniasis. We first asked whether IL-10 was able to inhibit IL-17 production. Peripheral blood mononuclear cells were stimulated with soluble leishmanial antigen (SLA) in the presence or absence of IL-10. After 4 days we harvested the supernatants and measured IL-17 levels. IL-17 was detected in the supernatants of cells from 13 out of 19 cutaneous, and 6 out 7 mucosal, patients (Figure 5E). When IL-10 was included in the culture, there was a significant decrease in IL-17 production by cells from both types of patients (Figure 5E). To determine if IFN-γ inhibited IL-17 production in human leishmaniasis, we stimulated cells from leishmaniasis patients with SLA in the presence or absence of anti-IFN-γ monoclonal antibody. In these experiments 8 out of 9 patients produced more IL-17 when anti-IFN-γ was included in the cultures (Figure 5F). Thus, taken together, our studies with cells from leishmaniasis patients indicate that similar to the murine model, both IL-10 and IFN-γ inhibit IL-17 production. Since there was an association with increased IL-17 and pathology when IFN-γ was neutralized in IL10SD mice, we hypothesized that neutralization of IL-17 would reverse the pathology observed in IL10SD mice. Mice were treated with anti-IL-10R mAb, anti-IL-17 mAb or both simultaneously, and lesion development was monitored. While IL-10SD mice exhibited larger lesions, there was a significant reduction in lesion size when IL10SD mice were treated with anti-IL-17 mAb (Figure 6A). Of note, neutralization of IL-17 had no effect on parasite numbers (Figure 6B). We observed that the number of neutrophils decreased in anti-IL-17 mAb treated IL-10SD mice in comparison to IL-10SD mice (Figure 6C). The antigen-specific IL-17 production by draining lymph node cells from anti-IL-17 mAb treated IL10SD mice was less than those from IL-10SD mice (Figure 6D), while IFN-γ responses were unaffected (Figure 6E). Similar results were observed when Il10−/− mice were treated with anti-IL-17 (Figure 6F). To confirm these results we examined the histology of the lesions from the mice where IL-17 was neutralized, and observed that lesions from IL10SD mice treated with anti-IL-17 mAb were similar in cell composition to isotype treated controls. As described above (Figure 1), IL10SD mice developed severe disease associated with substantial neutrophil infiltration, while in contrast both anti-IL-17 treated mice and IL10SD mice where IL-17 was neutralized appeared histologically similar to the isotype controls (Figure 6G). To determine what cytokines might be promoting the increased IL-17 responses in IL10SD mice, we looked at gene expression of IL-6, IL-23, TGFβ1 and IL-1β 1 week after infection of control and IL10SD mice. IL-1β was the only IL-17 associated cytokine gene that increased significantly after anti-IL-10R treatment (Figure 7A). Therefore, we examined the contribution of IL-1 in the pathology seen in IL-10SD mice by infecting Il1r1−/− mice and treating them with anti-IL-10R mAb. Il1r1−/− mice exhibited an identical course of infection as wild-type controls, consistent with previous studies [29]. However, in contrast to wild type mice treated with anti-IL-10R mAb, anti-IL-10R mAb treated Il1r1−/− mice developed lesions that were identical to C57BL/6 or Il1r1−/− isotype treated mice (Figure 7B), although they were better able to control the parasites (Figure 7C). Moreover, anti-IL-10R mAb treated Il1r1−/− mice failed to show the increase in infiltrating neutrophils that was seen in IL10SD mice (Figure 7D). Strikingly, while L. major-specific secretion of IFN-γ was just partially affected, L. major-specific IL-17 production was completely abrogated in anti-IL-10R mAb treated Il1r1−/− mice, (Figure 7E). Our results are consistent with studies indicating an important role for IL-1β in several models of IL-17-mediated disease [28], [41]–[44], and suggest that IL-1 plays a critical role in inducing IL-17 mediated pathology in leishmaniasis.

Leishmaniasis is a disease that exhibits a wide spectrum of clinical manifestations, from healing to non-healing cutaneous lesions to fatal visceral infections. The immune response is critical in controlling these parasites, but can also promote increased pathology. This is most evident in patients from South America, some of whom develop secondary lesions in the nasopharyngeal region that leads to severe disease. This disease, termed mucosal (or mucocutaneous) leishmaniasis is often non-responsive to therapy, and patients may have the disease for years. The hallmark of the infection is a very strong immune response as indicated by high IFN-γ and TNF-α production, but very few parasites present within the lesions [2]–[4]. Importantly, cells from these patients appear to produce less IL-10 when stimulated with leishmanial antigens than cells from self-healing patients, and within the lesions there is low expression of the IL-10 receptor [2], [7]–[9]. Thus, it is presumed that a lack of regulatory mechanisms in these individuals leads to the development of an over exuberant Th1 response. In order to study the factors that contribute to the immunopathology associated with an unregulated immune response to Leishmania, we treated L. major infected C57BL/6 mice with anti-IL-10R mAb (IL10SD) and monitored the course of infection with L. major. Using this model we are the first to demonstrate that IL-10 can play a critical role in controlling pathology in cutaneous leishmaniasis. The importance of IL-10 in regulating potentially immunopathologic responses is well documented [45], [46]. Mice lacking IL-10 develop severe colitis, demonstrating that IL-10 maintains control over the immune response to the normal bacterial flora in the gut [47]. Infection of IL-10 deficient mice with T. gondii or T. cruzi leads to increased control of the pathogen, but simultaneous increased pathology [48]–[50]. In leishmaniasis, the absence of IL-10 can lead to significantly better clearance of the parasites in both cutaneous and visceral leishmaniasis [23], [51], [52]. In fact, following low-dose infection with L. major the parasites can be completely cleared [51]. Similarly, we find many fewer parasites in both IL10SD and Il10−/− mice compared with wild-type mice. What has not been previously observed, however, is an increase in pathology in L. major infected IL-10 deficient mice. This could be explained by the low doses of L. major that were used in prior studies, since low doses of L. major in mice are associated with an immunologically silent phase, even in the absence of IL-10. In this situation parasites may be eliminated before a potentially immunopathologic response develops, while this is not the case with high parasite doses. Thus, it is important to point out that our model has the limitation that it required an initial high dose of parasites to promote pathology. In patients, however, factors other than parasite dose may be important in promoting pathology, such as the genetic background of the patient, the influence of the vector, the site of infection, the microflora in the skin, and/or the species/strain of the parasite, any of which may contribute to a much more pronounced early immune response after a natural infection [32], [53]–[55]. One dramatic example of this occurs in L. braziliensis infected patients, who develop substantial lymphadenopathy in the lymph node draining the infection site, often even before the lesion is evident [56], [57]. How a strong early immune response shapes the development of immunopathology at later time points is unknown. However, the larger the pool of Leishmania-experienced cells, the greater one might expect the pathology to be if regulatory mechanisms are deficient. Indeed, our findings suggest that while therapeutic blockade of IL-10 may decrease parasite numbers, under certain conditions it may also enhance disease. IL-10 is produced by many cell types, including T cells, B cells, and most myeloid-lineage cells [58]. In leishmaniasis, macrophages, CD4+ T regulatory cells and CD4+ Th1 cells produce IL-10, although the relative importance of each of these sources is debated and may change as the infection progresses [23], [30], [31]. IL10SD mice exhibit significant changes in the immune response soon after infection indicating that IL-10 plays a role from the initial phase of the infection. In our studies, we found that CD4+ (Foxp3+ and Foxp3−) and CD8+ T cells, along with some NK cells and myeloid cells (CD11b+), were the major IL-10 producers early after infection. No studies have identified the cells making IL-10 within the lesions of cutaneous leishmaniasis patients, although within the peripheral blood monocytes and regulatory T cells were identified as IL-10 producers [7], [59], [60]. In visceral leishmaniasis, Foxp3− T cells were found to be the predominant IL-10 producers in the spleen [52]. We are currently developing methods to analyze the cells within leishmanial lesions from patients, which will allow us to identify the IL-10 producing cells that are important in controlling immune mediated pathology at the infection site. The factors responsible for stimulating IL-10 vary depending upon the cell. Several TLR ligands induce IL-10 production by macrophages and dendritic cells, including TLR9, when stimulated by L. major parasites [61]–[63]. In addition, antibody opsonized Leishmania parasites, in conjunction with other signals, stimulate the production of IL-10 by macrophages via the FcγR [23]. This pathway may be more important at later stages of the infection once antibody responses have developed, which may explain the low level of IL-10 produced by myeloid cells at 1 week of infection. The factors that promote T cell IL-10 production are less well defined, but appear to involve strong antigenic stimulation and IL-12 production, both of which may contribute to the IL-10 production that we see in Th1 cells after a high dose infection with L. major [33], [58]. IL-27 also stimulates IL-10 production by CD4+ T cells, and Il27r−/− mice are more susceptible to L. major than wild-type controls [24]. Similar to the results presented here, Il27r−/− mice not only develop more severe lesions, but also increased IL-17 responses. However, in contrast to the response we see in IL10SD mice, Il27r−/− mice show an increase in IL-4 and a decrease in IFN-γ levels [24], [25]. Nevertheless, since IL-27 can directly regulate IL-17 responses [64]–[67], further study on its role in both experimental and human leishmaniasis is warranted. Our results demonstrate that regulating IL-17 production is critical for controlling Leishmania induced pathology. Thus, when IL-10 fails to control the immune response, there is a significant increase in IL-17 production. By neutralizing IL-17 we found that IL-17 was the major factor responsible for the increased pathology observed when IL-10R is blocked. To our knowledge this is the first demonstration that regulation of IL-17 during a Th1 response is critical in controlling Leishmania-induced immunopathology. A previous study in L. major infected Il17−/− BALB/c mice also showed that IL-17 was associated with more pathology, and in these animals the pathology developed in the context of a Th2 response [18]. Our results extend those studies by demonstrating that IL-17 also contributes to pathology in the context of a Th1 response. The role of IL-17 in human leishmaniasis has yet to be fully evaluated. In visceral leishmaniasis IL-17 has been associated with protection, and similarly in subclinical patients in Brazil IL-17 responses were elevated, suggesting a potential protective role [20]. Conversely, we found an increase in IL-17 production in patients infected with L. braziliensis, and there was a direct correlation between the magnitude of the cellular infiltrate and IL-17 [16]. Similarly, IL-17 levels were elevated in L. braziliensis infected patients with disease, but not patients who have resolved their infections [68]. Finally, an association with increased IL-17 and neutrophils has been observed in mucosal patients [17]. Indeed, in the data presented here there was a high IL-17 production by PBMC from mucosal patients compared with cutaneous Leishmania infected patients. Taken together, these results suggest that IL-17 contributes to disease in cutaneous leishmaniasis patients. Thus, understanding how to modulate IL-17 responses in patients may be important in controlling the worst aspects of the disease. As is the case for the differentiation of all CD4+ T helper cell subsets, the development and maintenance of Th17 cells is dependent upon the a specific combinations of cytokines, that can include TGF-β, IL-6, IL-23 and IL-1β [26], [41], [69]–[71]. However, we found the largest change in expression in IL-1β, and therefore asked whether Th17 cells, and the pathology associated with them, would be reduced in Il1r−/− mice. As has been previously reported, the course of infection with L. major in Il1r−/− mice was similar to control mice [29], [72]. However, it was striking that Il1r−/− mice treated with anti-IL-10R mAb failed to exhibit the increased pathology observed in treated wild-type mice. Correspondingly, these mice exhibited decreased levels of IL-17, as well as decreased infiltration of neutrophils when compared with IL10SD mice. These results suggest not only that the increased pathology we see in IL10SD mice is associated with IL-1, but also that IL-1 is required for the increased IL-17 expression observed in IL10SD mice. These findings also raise the possibility that treatment with an IL-1R antagonist may be therapeutic in certain forms of severe disease [73], [74]. Several mechanisms are described that operate to control the immune response, and in mucosal leishmaniasis an overproduction of IFN-γ was thought to be one factor promoting increased pathology. While we see increased IFN-γ when IL-10 is not regulating the response, our studies demonstrate that IL-17, rather than IFN-γ, contributes to the inflammation and tissue damage that occurs in the absence of IL-10 (Figure S3). We also show that IL-1β is a critical factor promoting the development of IL-17 producing T cells and neutrophil infiltration. Finally, we show that IL-10 and IFN-γ regulate the IL-17 responses of cells from human patients with leishmaniasis. Thus, this study demonstrates that the IL-17 pathway might be an important therapeutic target for the treatment of severe leishmaniasis in patients where the IL-10 regulatory function is compromised.

This study was conducted according to the principles specified in the Declaration of Helsinki and under local ethical guidelines (Ethical Committee of the Maternidade Climerio de Oliveira, Salvador, Bahia, Brazil). This study was approved by the Ethical Committee of the Federal University of Bahia (Salvador, Bahia, Brazil). All patients provided written informed consent for the collection of samples and subsequent analysis. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee, University of Pennsylvania Animal Welfare Assurance Number A3079-01. Female C57BL/6 mice 6–8 weeks old were purchased from the National Cancer Institute (Frederick, MD). B6. 129S7-Ilr1 tm1Imx/J (Il1r1−/−), B6 (Cg) -Il10 tm1. 1karp/J (Vert-X), expressing an IL-10-eGFP reporter, B6. 129P2- Il10 tm1. 1Cgn/J (Il10−/−) and B6. 129S7-Rag1tm1Mom/J (Rag1−/−) mice, were purchased from The Jackson Laboratory (Bar Harbor, ME). Mutant Il1r1−/−, ll10−/− and Rag1−/− mice were backcrossed to C57BL/6 genetic background for 5,13 and 10 generations to create each strain respectively. All mice were maintained in specific pathogen-free facilities at the University of Pennsylvania. All procedures were performed in accordance with the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC). Mice received monoclonal antibodies (mAbs) against IL-10R (250–500 µg; clone 1B1. 3A), IL-17A (500 µg: clone 17F3) (BioXcell, West Lebanon, NH.) or IFN-γ (1 mg; clone XMG-1. 2) 1 day prior to infection and twice a week thereafter. IL-10R mAb was used at a dose of 500 µg per mouse for the first two injections. L. major (WHO/MHOM/IL/80/Friedlin) promastigotes were grown to the stationary phase in Schneider' s Drosophila medium (GIBCO BRL, Grand Island, NY, USA) supplemented with 20% heat-inactivated fetal bovine serum (FBS, Invitrogen USA), 2 mM l-glutamine, 100 U of penicillin and 100 µg of streptomycin per mL. Infective-stage promastigotes (metacyclics) were isolated from 4–5 day old stationary culture by density gradient separation by Ficoll (Sigma) [75]. Mice were inoculated intradermally in the ear with 10 uL of PBS containing 2×106 L. major metacyclic promastigotes. Lesion development was measured weekly by ear thickness with a digital caliper (Fisher Scientific). Parasite burden in lesion tissues was assessed using a limiting dilution assay as previously described [76]. Freeze-thawed antigen (FTAg) was obtained from stationary-phase promastigotes of L. major. Soluble leishmanial antigen (SLA) was prepared from L. braziliensis parasites are previously described [77]. This study included patients with cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML). All patients were seen at the health post in Corte de Pedra, Bahia, Brazil, which is a well-known area of L. braziliensis transmission. The criteria for diagnosis were a clinical picture characteristic of CL and ML in conjunction with parasite isolation or a positive delayed-type hypersensitivity response to Leishmania antigen, plus histological features of CL and ML. In all cases, the immunological analysis was performed before therapy. This research was conducted with the approval of the Ethical Committee of the Maternidade Climerio de Oliveira (Salvador, Bahia, Brazil), and informed consent was obtained from each participant. For cell culture and IL-17 measurement, peripheral blood mononuclear cells (PBMCs) were obtained from heparinized venous blood layered over a Ficoll-Hypaque gradient (GE Healthcare), then washed and resuspended in RPMI1640 complete medium with 10% heat inactivated human AB serum (Sigma) at a concentration of 3×106 cells/mL. These cells were added to 24-well plates and were kept unstimulated or were stimulated with soluble leishmania antigen (5 ug/mL) for 96 h at 37C in 5% CO2. In some experiments recombinant human IL-10 (10 ng/mL) or anti- IFN-γ mAb (100 ug/mL, clone K3. 53) (R&D systems) was added. The supernatants were collected and stored frozen until analyzed for cytokines. IL-17 was measured by enzyme-linked immunosorbent assay (R&D Systems). The dorsal and ventral sides of mouse ear were split mechanically and placed dermis side down in a 24 wells plate in incomplete RPMI 1640 containing 0. 25 mg/mL of Liberase TL (Roche, Diagnostics Corp.) and 10 µg/mL DNase I (Sigma-Aldrich). Ears were incubated for 90 min at 37°C in a 24-well plate. Dermal cell suspensions were prepared by dissociation on 70-um cell strainer (Falcon) in PBS containing 0. 05% BSA and 20 µM EDTA. Single cell suspensions from the ear were obtained as described above. For analysis of surface markers and intracellular cytokines, some cells were incubated for 4 h with 10 µg/mL of brefeldin A, 50 ng/mL of PMA and 500 ng/mL ionomycin (Sigma-Aldrich). Before staining, cells were incubated with an anti-Fcγ III/II receptor and 10% rat-IgG in PBS containing 0. 1% BSA. Cells were stained for dead cells (Invitrogen) and surface markers [CD4, CD8β (BioLegend), CD45, CD90. 2, TCRγδ, NK1. 1, CD19, TCRαβ, Ly6G, CD11b, CD11c (eBioscience, San Diego, CA) Ly6C, MHCII (BDbioscience) ] followed by fixation with 2% of formaldehyde. For intracellular staining, cells were previously permeabilized with 0. 2% of saponin buffer and stained for IFN-γ, IL-17A, GFP (eBioscience, San Diego, CA), AF488 conjugated anti-rabbit Ab (Invitrogen, USA) or iNOS/NOSII (Millipore). Foxp3 staining was performed as indicated by Foxp3 kit (BD Bioscience). The data were collected using LSRII flow cytometer (BD) and analyzed using FlowJo software (Tree Star). For measurements of antigen-specific cytokine production in the mouse, the retroauricular lymph node was removed, mechanically dissociated, and single cell suspensions were prepared. Cells were resuspended in complete RPMI 1640 supplemented with 10% of FBS, 2 mM l-glutamine, 100 U of penicillin and 100 µg of streptomycin per mL and 0. 05 µM of β-mercaptoethanol. 4×106 cells per mL were plated in 24-well plates. Cells were incubated at 37°C in 5% CO2 with 20×106 L. major FTAg/mL. Supernatants were harvested 72 h after stimulation and assayed using standard IL-17A, IFN-γ and IL-4 sandwich enzyme-linked immunosorbent assay (ELISA) using commercially available antibodies (eBioscience, San Diego, CA). Cytokine concentrations were calculated from standard curves with detection limit of 0. 030 ng/mL for IFN-γ, 0. 015 ng/mL for IL-17A and 7 Units/mL of IL-4. Mice were sacrificed on specified days following infection; the ears were removed, fixed in 10% buffered formalin, and embedded in paraffin. Longitudinal 5 µm sections were cut and stained with hematoxylin and eosin. Photographs were taken with a Nikon Digital Sight DS-Fi1 Color system, (Nikon eclipse E600 Microscope). Epidermal thickness was measured on hematoxylin and eosin stained sections. Epidermal thickness was defined as the distance between the basement lamina and the apical surface of the uppermost nucleated keratinocytes, i. e. , the border between the stratum granulosum and stratum corneum. Total RNA was extracted from ear tissue samples in 1 ml TRIZOL reagent (Invitrogen). The sample was homogenized using a tissue homogenizer (FastPrep-24, MP Biomedical), and total RNA was extracted according to the recommendations of the manufacturer and further purified using the RNeasy Mini kit (QIAGEN). RNA was reverse transcribed using high capacity cDNA Reverse Transcription (Applied Biosystems). Real-time RT-PCR was performed on a ViiA™ 7 Real-Time PCR System (Applied Biosystems). Relative quantities of mRNA for several genes was determined using SYBR Green PCR Master Mix (Applied Biosystems) and by the comparative threshold cycle method, as described by the manufacturer. mRNA levels for each sample were normalized to Ribosomal protein S14 genes (RPSII) and displayed as fold induction over naïve or uninfected controls. Primers were designed using Primer Express software (version 2. 0; Applied Biosystems); RpsII, forward, 3′ CGTGACGAAGATGAAGATGC 5′ and reverse 5′- GCACATTGAATCGCACAGTC-3′); Il1b, forward, 5′- TTGACGGACCCCAAAAGAT -3′, and reverse, 5′- GATGTGCTGCTGCGAGATT-3′; Tgfb1, forward, 5′-CGCTGCTACTGCAAGTCAGA-3′ and reverse, 5′-GGTAGCGATCGAGTGTCCA-3′; Il23p19, forward, 5′-CCTAGGAGTAGCAGTCCTGA-3′, and reverse, 5′-TGCATGTGCGTTCCAGGCTA-3′; Il6, forward, 5′-ACAGAAGGAGTGGCTAAGGA-3′ and reverse, 5′-CACCATGGAGCAGCTCAG- 3′. Results represent means ± SEM. Data were analyzed using Prism 5. 0 (GraphPad Software, San Diego, CA). Statistical significance was determined by one-way ANOVA and by an unpaired two-tailed Student' s t test to compare means of lesion sizes, parasite burdens, and cytokine production from different groups of mice. For the experiments where human recombinant IL-10 or anti- IFN-γ was added, paired Wilcoxon test was used. Statistically significant differences were defined as \* when p values<0. 05.

Original summary:

Leishmaniasis is a tropical disease transmitted by sand flies that causes visceral and cutaneous lesions. In humans, the most severe form of cutaneous leishmaniasis is the mucosal form, causing disfiguring lesions in the nasal and oral mucosa. Why these patients develop severe disease is not clear. It is known, however, that the severe disease is not due to an overwhelming number of parasites, but rather appears to be due to an uncontrolled inflammatory response that includes elevated production of IFN-γ and IL-17. Here, we used a murine model of leishmaniasis to identify the factors involved in this pathology, and found that mice infected with Leishmania major developed severe lesions in the absence of IL-10 or IL-10 signaling, and similar to patients, contained high levels of IFN-γ and IL-17. While both of these cytokines have the potential to induce pathology, we found that IL-17 was responsible for the severe pathology seen in the absence of IL-10 regulation, and furthermore that IL-17 levels were higher and pathology greater in the absence of IFN-γ. Thus, our study suggests that IL-17, but not the IFN-γ, is a strong candidate to be targeted in strategies to control the severe immunopathology observed in mucosal leishmaniasis patients.

Predicted:

Leishmania is a protozoan parasite that consists of a wide spectrum of clinical manifestations, from healing cutaneous lesions to fatal visceral infections. This disease, termed mucosal leishmaniasis, exhibits decreased IL-10 levels and an exaggerated inflammatory response that perpetuates the disease. Using a mouse model, we investigated what cytokines contribute to increased pathology when IL-10-mediated regulation is absent. Leishmania major infected C57BL6 mice lacking IL-10 regulation developed larger lesions than controls, but fewer parasites. Both IFN- and IL-17 levels were substantially elevated in mice lacking the capacity to respond to IL-10. We found that IFN- promoted an increased infiltration of monocytes, while IL-17 contributed to an increase in neutrophils. These observations suggest that the lack of IL-10 or responsiveness to IL-10 may be an important contributing factor in the immunopathology observed in this disease. A better understanding of the pathogenesis of mucosal disease is important, since drug therapy is often not successful in these patients 1013. It is believed that high levels of IFN- and TNF- contribute to the disease, which might provide targets for immunotherapy 14, 15. More recently, increased levels of IL-17 have been identified in patients with cutaneous and mucosal lesishmaniais, suggesting that IL-17 may also play a pro-inflammatory role in this disease and could be a target for immunotherapy 16, 17. Moreover, BALBc mice lacking IL-17 develop significantly smaller lesions than control mice, and also exhibit reduced IL-10 levels concomitant with a Th2 response that may also contribute to the disease 24, 25. Therefore, we propose that regulating IL-17, possibly by downregulating IL-1, may be a useful approach for controlling immunopathology in lesishmaniasis. We found that IL-10 was primarily produced by CD4Foxp3 and CD4 Foxp3 cells. As expected, there was a substantial increase in the production of IFN- in IL10SD mice early after infection, which mediated both increased recruitment of monocytes as well as iNOS production. We also found a significant increase in IL-17 production and neutrophil accumulation in the lesions of IL10SD mouse. Therefore, we next addressed the question of whether IFN- or IL-17 or both was required for the development of immunopathology in the absence of IL-10 signaling. We found that Il1r1 mice failed to develop severe pathology when treated with anti-IL-10R mAb, and correspondingly showed reduced IL-17 levels and the lack of neutrophil recruitment.

{'rouge1': Score(precision=0.36724565756823824, recall=0.7115384615384616, fmeasure=0.4844517184942717), 'rouge2': Score(precision=0.13432835820895522, recall=0.2608695652173913, fmeasure=0.1773399014778325), 'rougeL': Score(precision=0.1488833746898263, recall=0.28846153846153844, fmeasure=0.19639934533551556)}

(ds['test']['summary'][1000])

Input:

Human T-Lymphotropic Virus type 1 (HTLV-1) is a human oncoretrovirus that infects at least 5 to 10 million people worldwide and is associated with severe diseases. Africa appears as the largest HTLV-1 endemic area. However, the risk factors for the acquisition of HTLV-1 remain poorly understood in Central Africa. We conducted an epidemiological survey between 2013 and 2017, in rural areas of 6 provinces of Gabon, in a rainforest environment. Epidemiological data were obtained and blood samples were collected after informed consent. Plasma were screened for HTLV-1 antibodies by ELISA and the positive samples were then tested by Western blot (WB). Genomic DNA derived from buffy-coat was subjected to two semi-nested PCRs amplifying either HTLV-1 env gene or LTR region fragments. We recruited 2,060 individuals over 15 years old, including 1,205 men and 855 women (mean age: 49 years). Of these, 299 were found to be ELISA HTLV-1/2 seropositive. According to WB criteria, 136 were HTLV-1 (6. 6%), 25 HTLV-1/2 (1. 2%) and 9 HTLV seroreactive (0. 4%). PCR results showed that 146 individuals were positive for at least one PCR: 104 for the env gene and 131 for the LTR region. Based on both serological and molecular results, 179 individuals were considered infected with HTLV-1, leading to an overall prevalence of 8. 7%. The distribution of HTLV-1 infection was heterogeneous across the country. Based on multivariable analyses, female gender, increasing age, ethnicity (Pygmy) and multiple hospitalizations (more than 5 times) were found to be independent risk factors for HTLV-1 infection. Furthermore, a non-human primate bite appeared to be marginally associated with a higher risk of HTLV-1 infection. Based on state-of-the-art serological and molecular methods, we have demonstrated that rural adult populations in Gabon are highly endemic for HTLV-1. Our results regarding risk factors should lead to public health actions aiming to reduce HTLV-1 transmission.

Human T-lymphotropic virus type 1 (HTLV-1), the first human retrovirus discovered [1], is the etiological agent of several pathologies, mainly a very severe T-cell lymphoproliferation named Adult T-Cell Leukemia Lymphoma (ATLL) and a chronic disabling neuro-myelopathy, the Tropical Spastic Paraparesis/HTLV-1 Associated Myelopathy (TSP/HAM) [1–4]. HTLV-1 is not ubiquitously distributed worldwide. Indeed, it is mainly present in foci where viral prevalence can reach 2 to 40% in adults, depending on age, sex and geography. The most important HTLV-1 endemic areas are: the Southern part of the Japanese archipelago, several areas in South America and the Caribbean basin as well as some areas of Australo-Melanesia, Iran and large regions of sub-Saharan Africa. This human oncoretrovirus is estimated to infect at least 5 to 10 million people worldwide [5,6]. While the great majority of HTLV-1 infected individuals remains asymptomatic throughout their life, ATLL and TSP/HAM occur in 2 to 7% of them [7]. The three main routes of HTLV-1 transmission are: mother-to-child through prolonged breastfeeding (mostly over 6 months) [8], sexual (mainly from male to female) [9] and by blood products contaminated with infected cells [10]. HTLV-1 originates from its simian counterpart STLV-1, which is highly prevalent in several Non-Human Primates (NHPs) species. Zoonotic transmission of STLV-1 still occurs mainly through severe NHP bites, at least in Central and West Africa [11,12]. Sub-Saharan Africa is considered as the largest HTLV-1 endemic area accounting for at least half of the infected individuals worldwide (2. 5 to 5 millions). However, the situation of HTLV-1 in Africa is not well known. Indeed, the majority of previous studies have been carried out either on very specific populations such as pregnant women, blood donors or hospitalized patient series, or in heterogeneous and relatively small groups of rural or urban inhabitants of a specific town or village or area [5,13]. All these groups are far from being representative of the population of a given region or country. Furthermore, many of these prevalence studies only include serological analyses, without molecular detection of HTLV-1 proviral DNA. Lastly, there are still few epidemiological studies investigating HTLV-1 acquisition risk factors in the African continent [14–17]. Gabon, located in Central Africa, appears to be highly endemic for HTLV-1 and is an appropriate area for the study of HTLV-1 acquisition risk factors [18–21]. Indeed, to date, no public health action has been undertaken to prevent dissemination of this virus in Gabon, while the four modes of transmission are still prevalent in this country. In this work, we performed a large epidemiological study among adult inhabitants of rural Gabon. We used state-of-the-art assays for both serological and molecular diagnoses. Our first objective was to provide better knowledge of the prevalence and the geographical distribution of HTLV-1 infection in Gabon. The second was to get new insights into the risk factors for the acquisition of HTLV-1 among adults living in this highly endemic area.

Between 2013 and 2017, we conducted epidemiological surveys involving inhabitants of rural areas living mainly in a rainforest environment, in 6 out the 9 provinces of Gabon. A systematic approach for the enrolment of adults (over 15 years old) was carried out in the populations from all reachable villages and settlements, scattered along side roads and tracks across the primary tropical forest. We included all volunteers. A standardized questionnaire was used to collect epidemiological data. Besides demographic information, we focused our questions on the potential risk factors for HTLV-1 acquisition including scarification, hospitalization, transfusion and contacts with wild animals and bush meat during hunting and/or butchering activities (especially with monkeys and apes). A 5 to 10 ml blood sample was collected on EDTA from all consenting individuals. Plasma and buffy coat were obtained 48 to 72 h after sampling and kept frozen at -80°C. Ethical approval was obtained from the Comité National d’Ethique of Gabon (Permit number PROT 0011/2013/SG/CNE). Prior to field sampling, individual written informed consent was obtained from all participants after detailed information and explanations of the study were provided to the community. Written informed consent for children was obtained from their parents or recognized guardians. Plasma samples were screened for HTLV-1/2 antibodies by ELISA tests (HTLV-I/II ELISA 4. 0, MP Biomedicals), and confirmatory Western blot assays (HTLV BLOT 2. 4, MP Biomedicals) for all ELISA positive samples. Results were interpreted according to the manufacturer’s instructions. Thus, the samples were classified as HTLV-1 seropositive, HTLV-2 seropositive, both HTLV-1/2 seroreactive, HTLV seroreactive, indeterminate and seronegative. High molecular weight DNA was extracted from peripheral blood buffy coats (PB-BC) using the QIAamp blood minikit (Qiagen). DNA samples were determined as amplifiable after a polymerase chain reaction (PCR) positive result, performed on the human β-globin gene. One microgram of each DNA sample was then tested by two different semi-nested PCRs: according to protocols previously described [22]: a first one to amplify a 522-bp fragment of the env gene, and a second one to amplify a 450-bp fragment of the LTR-3’ region of HTLV-1. A sample was considered as PCR positive when a band of the expected size was clearly detected on agar gel. An individual was considered as infected with HTLV-1 if the WB profile was either HTLV-1, or HTLV-1/2 or HTLV. Furthermore, an individual with an indeterminate WB profile associated with at least one HTLV-1 positive PCR, was also considered as HTLV-1 infected. Age was compared between Pygmy and Bantu ethnic groups using the Student t test. HTLV-1 infection prevalence was compared between provinces using the Chi-square test, and between age groups using the Chi-square test for trends. Logistic regression was used to identify factors associated with HTLV-1 infection and to estimate odds ratios (OR) and 95% confidence intervals (95%CI). ORs were presented as crude or age and gender adjusted (univariable analysis) and all variables adjusted (multivariable analysis). Variables retained in the final model were considered statistically significant if p values were < 0. 05, and marginally significant if p values were < 0. 07. Multiple imputations were performed to replace missing values for the “number of hospitalizations” variable, using the multivariable imputation by chained equations (MICE) method [23]. All analyses were performed using STATA 15. 0 software (Stata Corporation, College Station, TX, USA).

Among the 2,060 plasma samples tested by ELISA, 299 were found HTLV-1/2 seropositive. Using WB criteria provided by the manufacturer, 136 were HTLV-1 seropositive (6. 6%), 25 HTLV-1/2 (1. 2%), 10 HTLV-2 (0. 5%), 9 HTLV seroreactive (0. 4%) and 85 sero-indeterminate (4. 1%). Moreover, 34 were seronegative. Based on strict WB results as defined by the manufacturer’s assay (HTLV Blot 2. 4, MP Biomedicals) and including only HTLV-1 and HTLV-1/2 profiles, the overall HTLV-1 seroprevalence was 7. 8% (95%CI 6. 7–9. 1,161/2,060) (Table 1). Molecular amplification was performed on DNA extracted from the PB-BC of 299 ELISA positive individuals. The results showed that 146 were positive for at least one PCR (104 for the env gene, 131 for the LTR region), and 89 for both PCRs (Table 1). Based on both serological and molecular results, 179 individuals were considered as HTLV-1 infected. This includes: i) the 170 individuals with a WB profile being either HTLV-1, HTLV-1/2 or HTLV, and ii) the 9 individuals with an indeterminate WB profile associated with at least one positive HTLV-1 PCR test (Table 1). Thus, 179 HTLV-1 infected persons were included in the epidemiological analysis (Fig 2A and 2B). This leads to an overall HTLV-1 infection prevalence of 8. 7% (95%CI 7. 5–10. 0). Univariable analysis revealed that women are significantly more likely to be HTLV-1 infected than men (age-adjusted OR 1. 69; 95%CI 1. 2–2. 3, Table 2). Indeed, 97 of 855 women (11. 35%) and 82 of 1,205 men (6. 8%) were HTLV-1 infected. As illustrated (Fig 2A and Table 2), the age specific seroprevalence gradually increases in the study population (p <0. 001), reaching 17. 3% among women of the 61 to 70 age group. Furthermore, Pygmy individuals are at increased risk of being HTLV-1 seropositive compared to Bantu people, after adjustment for age and sex (Adjusted OR 1. 67; 95%CI 1. 04–2. 68, Table 2 and Fig 2B). Individuals included in this study originate from 6 of the 9 Gabonese provinces (Fig 3). HTLV-1 seroprevalence varies according to the geographic origin of infected individuals (Fig 3 and Table 2). Ogooué-Ivindo and Ogooué-Lolo provinces present a significantly higher HTLV-1 prevalence (respectively 14% and 11%; p = 0. 027) compared to Ngounié, Woleu-Ntem and Nyanga provinces, where lower infection rates were recorded. Moreover, the Haut-Ogooué province shows an intermediate HTLV-1 seroprevalence (9. 5%), which is not significantly different from the other provinces (Fig 3 and Table 2). Indicators for HTLV-1 parenteral transmission were also assessed. According to crude OR, HTLV-1 infection is associated with behaviors leading to scarifications (crude OR 1. 46; 95%CI 1. 04–2. 05), an history of blood transfusion (crude OR 1. 61; 95%CI 1. 06–2. 44), and an increased number of hospitalization (more than five times, crude OR 2. 96; 95%CI 1. 56–5. 62), but not with tattoo nor circumcision. However, when adjusted for age and sex, only individuals with 5 hospitalizations or more were at increased risk of being HTLV-1 infected (Table 2). Since our study was conducted in primary tropical forest areas and focused on individuals potentially at risk of acquiring HTLV-1 through interspecies transmission, we also obtained information on contacts with NHPs. No association was observed with hunting and butchering activities or keeping NHPs as pets. However, a higher risk was observed among individuals who reported having been bitten by a NHP. Indeed, 8 of 53 individuals (15. 1%) were HTLV-1 seropositive (adjusted OR 2. 31; 95%CI 1. 05–5. 11, Table 2). In the final multivariable analysis model (Table 3), we found an increased independent risk of HTLV-1 infection in women (OR 1. 61; 95%CI 1. 16–2. 23), the elderly people (e. g. >70; OR 3. 47; 95%CI 1. 89–6. 37), persons having a history of multiple hospitalizations (more than 5 times, OR 2. 38; 95%CI 1. 21–4. 66), and individuals belonging to the Pygmy ethnic group (OR 1. 93; 95%CI 1. 18–3. 14). In addition, a NHP bite appears to be marginally associated with a higher risk of HTLV-1 infection (OR 2. 16; 95%CI 0. 97–4. 8).

This study represents one of the largest epidemiological study conducted in Central Africa. We show that HTLV-1 is highly endemic among villagers living in the different provinces of the primary tropical forest of Gabon. These data confirm the high prevalence of HTLV-1 in Gabon and extend it to most rural areas, since we studied adult populations of both sexes, from 6 of the 9 rural provinces of this country. Previous studies mainly carried out in pregnant women or in populations from some regions of Gabon (mostly Franceville area and/or Eastern Gabon regions), have observed a high level of HTLV-1 infection [18–21,24,25]. However, such studies were mostly based on serological analyses with, for the oldest studies, Western blot interpretation criteria that are now considered as poorly specific. The results of our paper confirm the low specificity of the ELISA tests used for HTLV-1 serological screening. Furthermore, most of the indeterminate WB profiles (89. 4% in our study) were PCR negative in DNA extracted from blood. These findings are frequently observed in central Africa where some of these false positives correspond to non-specific cross-reactivities, particularly between HTLV-1 Gag proteins and antigens derived from malaria [26]. Here, on the basis of state-of-the-art serological and molecular assays, we confirm a high level of retroviral infection. Surprisingly, this prevalence (8. 7%) appears to be higher than in neighboring countries. Indeed, if we analyze the results obtained on similar populations (pregnant women or Bantu adults from rural areas of the rainforest), and using comparable assays, it is obvious that the overall level of HTLV-1 prevalence is at least 2 to 3 times higher in Gabon than in South Cameroun, in Congo or in Central African Republic [27–29]. The reasons for these differences remain to be elucidated. Of note, the existence of high prevalence foci close to low or very low endemic areas is a hallmark of HTLV-1 epidemiology. In South Japan, areas of low HTLV-1 prevalence can be very close to highly endemic areas, especially in rural regions. Furthermore, in a previous study performed in a geographically limited area of Eastern Gabon, the HTLV-1 seroprevalence rates greatly varies between villages, from 5 to 17. 8% [21]. In our study, we observed a significant increase in HTLV-1 infection with age, as well as a higher prevalence in women. Such findings are well-known major characteristics of HTLV-1 epidemiology, regardless the endemic level and the studied areas. For instance, this has already been observed in rural populations of Guinea Bissau [15,17], as well as in patients hospitalized in Cameroon [28] or in Benin [30]. Lastly, this increased prevalence in women and with age is commonly found in various populations, such as inhabitants of Salvador de Bahia in Brazil, workers in Jamaica [31] or urban inhabitants of Southern Japan [32]. Such consistency indicates that this is a major specificity of this virus. It has been considered that cumulative sexual transmission over the years, predominantly occurring from male to female, may explain this specificity [33]. In some cases, a cohort effect can also be partly responsible for such an increase with age, as demonstrated in blood donors in Japan [34]. Surprisingly, a higher HTLV-1 seroprevalence in women was not observed in two previous studies performed in Gabon [19,35]. These discrepancies might be related to the age of the studied population. Indeed, significant sex-specific differences in HTLV-1 prevalence are not found in young adult age groups [21,32,33,36]. Despite the small number of Pygmy individuals included in this study as compared to Bantu (261 vs 1,797), the prevalence of HTLV-1 is significantly higher in Pygmies. Besides, in our multivariate analysis, being a Pygmy was associated with an increased independent risk of HTLV-1 infection (OR 1. 93; 95%CI 1. 18–3. 34). Such findings were previously observed for HTLV-1 and also for HTLV-2 infection in Bakola’s Pygmies living in the Ocean region, located in the Western part of South Cameroon [37]. The reasons why these retroviral infections have a higher endemic level in Pygmy populations remain unclear. It has been hypothesized that it may be linked to a founder effect with persistent high level of viral transmission. This may either be due to specific intrafamilial activities (for example prolonged breastfeeding or breastfeeding of several children by the same woman…), or to behavioral factors such as initiation rites, leading to blood contamination by non-sterile needles or sharp objects such as razor blades. Some of these hypotheses have also been proposed for other populations highly endemic for HTLV-1 or HTLV-2, such as Indigenous Australians living in Central Australia [38] or Amerindians from South American countries [39]. Concerning the distribution of HTLV-1 prevalence in the different provinces of Gabon, our data indicate a heterogeneous distribution ranging from 5. 5% to 14%. Contrary to what was previously suggested, we have not observed a gradient of HTLV-1 prevalence following the North-South axis. Such discrepancy could be related to various factors including the non-homogeneous and therefore non-comparable structure of the populations previously studied (urban vs rural). In a previous study, the HTLV-1 age-adjusted prevalence rate was significantly higher in rural areas of Gabon than in urban areas [19]. Moreover, the gradient was especially suggested when looking at the population of pregnant women living in urban areas [24]. In this study, 15% of inhabitants bitten by a NHP were HTLV-1-infected compared to only 8. 5% for people not bitten by such primates. In addition, NHP bite appears to be marginally associated with a high risk of HTLV-1 infection in our multivariable analysis. These results are reminiscent of those recently published that demonstrated that the bite by a monkey is a risk factor for HTLV-1 infection in a population of hunters living in tropical forest villages of Southern Cameroon [12]. They also echo the recent case-report of a young girl from a village in Eastern Gabon, which showed likely interspecies transmission of STLV-1 upon a severe bite by a C. nictitans [40]. Lastly, several publications also indicate that simian foamy viruses (belonging to another family of simian retroviruses) are highly endemic in Central African monkeys and apes, and can easily be transmitted to humans by severe bites from a NHP, leading to chronic infection [41]. In our study, one other risk factor associated with HTLV-1 is hospitalization. Indeed, HTLV-1 prevalence increases from 6% to 17% with the number of hospital stays. In the multivariable analysis, there is an increased independent risk of HTLV-1 infection in individuals with a history of multiple hospitalizations (more than 5 times). The reasons for the existence of such a risk are not clear. It could be linked to the nosocomial acquisition of this retroviral infection in hospital, either by transfusion of cells infected with HTLV-1, or by contamination through the use of infected syringes or non-sterile utensils. However, transfusion is not associated with HTLV-1 infection in our model. One may suppose that HTLV-1 is not actually transmitted at the hospital. For instance, the virus could be acquired through severe bite, which could secondarily lead to hospitalization. Similarly, a given illness could lead the patient to first visit a traditional healer with risky practices (e. g. use of non sterile material), before heading to more classical hospitalization. We hoped that this study would alert Gabonese public health authorities and promote appropriate measures to prevent HTLV-1 infection throughout the country, at least in rural populations in which we reported high prevalence. Despite the fact that Gabon is both a highly HTLV-1 endemic area and a high development index country, it has not yet introduced routine screening for HTLV-1, especially among blood donors. Importantly, a previous study in Gabon has already demonstrated that transfusion is a major risk factor for HTLV-1 acquisition in children [16]. In addition, several studies around the world have clearly demonstrated that HAM/TSP is frequently linked to HTLV-1 acquisition of blood by transfusion of contaminated cells [42]. Routine testing of blood donors seems the most necessary and easiest measure to implement quickly. In this context, we are currently conducting a survey, in close collaboration with the Libreville blood bank, in order to get new insights in the prevalence of HTLV-1 among blood donors. Another control measure that could be implemented is the screening for HTLV-1 infection in pregnant women. However, this raises the delicate and complex issue of decreasing or stopping breastfeeding in HTLV-1 infected women in a context of resource-limited countries. Finally, public health information campaigns on the zoonotic risks associated with hunting monkeys are also necessary.

Actual:

Human T-Lymphotropic Virus type 1 (HTLV-1) infects at least 5 to 10 million people worldwide and is associated with severe diseases. Africa, particularly the sub-Saharan region, is considered as the largest HTLV-1 endemic area. HTLV-1 can be acquired through three main routes: mother-to-child, sexual and through contaminated blood products. In addition, zoonotic transmission from non-human primates still occurs in specific rural African populations. Gabon is considered to be a very high HTLV-1 endemic area. However, risk factors for such a retroviral infection remain poorly known in Central Africa. In this study, using state-of-the-art serological and molecular tests, we aimed to get new insights on risk factors associated with HTLV-1 infection in rural Gabon. We report an overall prevalence of HTLV-1 of 8. 7%, demonstrating that HTLV-1 is highly endemic in rural adult Gabonese populations. In the study population, we identified female gender, increasing age, ethnicity (Pygmy) and multiple hospitalizations (more than 5 times) as independent risk factors for HTLV-1 infection. Furthermore, a non-human primate bite appeared to be marginally associated with a higher risk of HTLV-1 infection. Our results regarding the risk factors for such a retroviral infection are important for public health actions aiming to reduce HTLV-1 transmission.

Predicted:

Human T-lymphotropic virus type 1 HTLV-1, the first human retrovirus discovered 1, is the etiological agent of several pathologies, mainly a very severe T-cell lymphoproliferation named Adult T-Cell Leukemia Lymphoma ATLL and a chronic disabling neuro-myelopathy, the Tropical Spastic ParaparesisHTLV-1 Associated Myelopathy TSPHAM 14. HTLV-1 is not ubiquitously distributed worldwide. Indeed, it is mainly present in foci where viral prevalence can reach 2 to 40 in adults, depending on age, sex and geography. The most important HTLV-1 endemic areas are: the Southern part of the Japanese archipelago, several areas in South America and the Caribbean basin as well as some areas of Australo-Melanesia, Iran and large regions of sub-Saharan Africa. This human oncoretrovirus is estimated to infect at least 5 to 10 million people worldwide and is associated with severe diseases. However, the risk factors for the acquisition of HTLV-1 remain poorly understood in Central Africa. We conducted an epidemiological survey between 2013 and 2017, in rural areas of 6 provinces of Gabon, in a rainforest environment. Epidemiological data were obtained and blood samples were collected after informed consent. Plasma were screened for HTLV-1 antibodies by ELISA and the positive samples were then tested by Western blot WB. Genomic DNA derived from buffy-coat was subjected to two semi-nested PCRs amplifying either HTLV 1 env gene or LTR region fragments. We recruited 2,060 individuals over 15 years old, including 1,205 men and 855 women mean age: 49 years. Of these, 299 were found to be ELISA HTLV-12 seropositive. According to WB criteria, 136 were HTLV-1 6. 6, 25 HTLV-12 1. 2 and 9 HTLV seroreactive 0. 4. PCR results showed that 146 individuals were positive for at least one PCR: 104 for the env gene and 131 for the LTR region. Based on both serological and molecular results, 179 individuals were considered infected with HTLV-1. Our results regarding risk factors should lead to public health actions aiming to reduce HTLV-1 transmission.

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Patent

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BACKGROUND OF THE INVENTION This invention relates to a fuel economy running indicator which may be used during the driving operation of an automobile. In general, an internal combustion engine for a vehicle is designed to have a scope for operating at a constant gear ratio of speed change gears even if the load of the vehicle changes considerably. Accordingly, it is possible that a driver selects the gear ratio within the scope of a certain degree to operate a vehicle, such as a truck, having a number of speed change gears. However, under a constant power output, the internal combustion engine used for such a vehicle makes it possible to accomplish a satisfactory state of specific fuel consumption, that is, a fuel economy running state, by setting a suitable operation condition and, for this purpose, it is necessary to continually set a suitable gear ratio or proper travel of an accelerator pedal. This has become a substantial problem in this energy conserving age. Prior to the disclosure of the present invention, the explanation relating to economic fuel consumption will be generally given hereinafter. The specific fuel consumption increases gradually as the curves go outwardly. If the truck runs at about 90 Kmh on an express highway, the speed of the Diesel engine comes up to No. If No is constant, it defines a border line c and the output torque T of the engine is given by an operating point f. In a running state, an automobile must be repeatedly accelerated and decelerated not only in the suburbs but in the town. In the acceleration state, the operating points of the engine exist in the upper area of the running resistance characteristic i. As described above, in order to minimize the fuel cost in operation in consideration of a high vehicle speed as required on an express highway and a power enough to accelerate the vehicle, it is preferable to operate it within the area defined by border lines a, b and c. Notably, the border line a indicates an equal travelling amount characteristic of the accelerator pedal which is maintained in the state getting close to the minimum fuel consumption, and this state satisfies a significant increase of the torque T which is required to increase a tractive force of the vehicle. Also, the border line b means an operating state which is being accelerated from that of the increased tractive force, approaching the minimum fuel consumption as closely as possible, and indicates that, in the engine operation, it is desirable to reduce a travelling amount a 1 of the accelerator pedal, as the engine speed N increases. Further, the border line c indicates the limited maximum speed of the vehicle and also indicates that it is possible that No gets close to the area of the minimum fuel consumption, if the maximum speed of the vehicle is permitted to be relatively lowered, such as during the running in the town. SUMMARY OF THE INVENTION An object of the present invention is to provide a novel fuel economy running indicator. Another object of the present invention is to provide a fuel economy running indicator indicating whether a combination of an accelerator pedal travelling amount determining the power output state of an internal combustion engine and a gear ratio of speed change gears, which are selected from some operating parameters varied by a driver, is within the scope of an economy fuel consumption. These and other objects and features of the present invention will be better understood from the following description taken in conjunction with the accompanying drawings. A voltage at the neutral point of an AC generator is applied to an input terminal 1. The AC generator may be mounted on a Diesel engine of a truck and driven by the engine. A pulse-voltage converter 1a converts AC pulse signals at the input terminal 1 to a voltage level signal. A voltage signal proportional to a travelling amount of an accelerator pedal with the Diesel engine is applied to a further input terminal 3. Leads 2b and 3a are respectively connected to first and second inputs of a comparator 4. An output lead 4a of the comparator 4 is connected to a green lamp 5 through an amplifier 5a and is also connected to a red lamp 6 through an inverter 6a and an amplifier 6b. The operation of the construction as described above will now be described. The AC generator of the engine outputs a plurality of pulse signals proportional to the engine speed, which are applied to the input terminal 1, and the pulse signals are converted to a voltage level proportional to the number thereof. Thus, the voltage level on the lead 2a corresponds to the engine speed. The converted voltage value is compared with a voltage value corresponding to an accelerator pedal travelling amount, which is inputted through the lead 3a, by the comparator 4 which outputs a voltage signal on a lead 4a when the voltage value on the lead 2b becomes larger than that on the lead 3a. In this characteristic a 2 , when the voltage on the lead 3a corresponding to an accelerator pedal travelling amount is smaller than the output voltage V 2 corresponding to the characteristic a 2 , the comparator 4 generates a voltage at the output thereof. Accordingly, while the accelerator pedal is pushed down at the engine speed N exceeding No, the voltage value on the lead 3a is above zero on the lead 2b and, therefore, there is no voltage signal at the output of the comparator 4. According to the operation above, when the voltage signal is outputted to the lead 4a, the signal is amplified by the amplifier 5a and then the amplified signal illuminates the lamp 5. However, the lamp 6 is not energized because of the inverter 6a which is inserted between the lamp 6 and the comparator 4. Alternatively, when the voltage does not appear on the lead 4a, the lamp 5 is not energized while the signal is applied to the amplifier 6b because of the inverter 6a and then the amplified signal illuminates the lamp 6. As described above, in general, the driver of an automobile must continuously accelerate and decelerate, while watching obstacles in the forward direction. If the accelerator pedal is further pushed down at the normal state, the engine is accelerated and, therefore, the vehicle is also accelerated. If the operator releases slightly the accelerator pedal in obedience to the warning indication, the red lamp 6 turns off and the green lamp 5 turns on. Subsequently, if this pedal position is maintained as it is, the engine speed N increases gradually along the border line a and then exceeds the border line b to thereby turn on the red lamp 6. Thus, the operator again releases the accelerator pedal by degrees until the red lamp 6 turns off. This state is continued until the operating point of the engine follows the course from an arrow d 1 to d and reaches a normal operating state point j on the running resistance characteristic i. In the illustration above, since the weight of both the lead and the body itself of the truck is relatively heavy, the arrows d 1 and d change greatly in comparison with these of an automobile for riding. The operator, therefore, may have enough scope to control the travelling of the accelerator pedal, while paying attention to the indication of the lamps 5 and 6. This, of course, can be applied not only to a truck but also to a vehicle, such as a bus, used for carrying a number of passengers, and may also be applied to a general automobile for riding. The acceleration at climbing requires a change gear having a relatively low gear ratio to the over-top gear ratio as noted above and, in response to the selection of such a gear ratio, the operating scope is changed. However, since any operating point is indicated by the illumination of either the lamp 5 or 6, it is possible that the operator selects an efficient gear ratio as desired. The comparator 4 compares the output voltage V 2 with the voltage value corresponding to a travelling amount of the accelerator pedal. However, it is apparent that the border lines a, b and c may be presented as a function of the travelling amount and the presented reference output voltage is compared with a function of the true engine speed N. In the scope in which the travelling amount corresponds to the characteristic B 2 , the output signal on the lead 2b represents a reduced voltage value as the travelling amount increases and, when the output voltage is higher than a voltage value on the lead 3a, the red lamp 6 illuminates. When an amount of travelling the accelerator pedal, , exceeds the characteristic A 2 , the output voltage on the lead 2b, corresponding to a reference engine speed Ni, always becomes a zero value and, thus, the voltage value on the lead 3a is larger than the value on the lead 2b to thereby turn on the red lamp. Consequently, it is apparent that the comparator 4 may compare an engine speed and an amount of travelling the accelerator pedal, one of which has been characteristic-converted. In obedience to such warning indication, the driver can continuously control the engine such that the engine is operated within the area defined by the lines and, thus, the vehicle is permitted to repeat the accelerating operation, approaching the minimum fuel consumption rate as closely as possible. Accordingly, it is possible to reduce the fuel consumption within the limits of possibility, effecting the high-speed running required of an automobile and the repetition of the accelerating and decelerating operations. Further, since it is easy to change an amount of travelling of the accelerator pedal by merely judging the illumination of one of the green lamp 5 and the red lamp 6, the driver is not required to have any new operating techniques in addition to a conventional techniques. Still further, since the present invention is constructed by a very simple circuit such that the output signal of the characteristic converter 2 is compared with a detected signal other than the detected signal input to the converter 2, the unit cost of production is reduced. Additionally, according to the fuel economy running indicator of the present invention, it is possible to detect the engine speed at the neutral point of the A.C. generator without using an expensive device, such as an electromagnetic pickup. Therefore, the mounting cost of the overall system is economical.

Actual:

A fuel economy running indicator for an automobile, in particular, a truck with a Diesel engine, includes a characteristic converter for converting a first operating parameter of the engine to an output signal having a functional relationship with the first operating parameter, the functional relationship being such that the output signal represents a limited value in the scope which the first parameter has a relatively small value and the output signal always represents a zero value when the first parameter exceeds a predetermined value. The indicator also includes a comparator for comparing the output signal with a second operating parameter of the engine and for generating an output signal in response to the magnitude comparison thereof. The indicator further includes an indicator which operates in response to the output signal of the comparator for indicating whether or not the operation of the engine is within the scope of an economic fuel consumption rate.

Predicted:

the present invention provides a fuel economy running indicator which may be used during the driving operation of an automobile . under a constant power output , an internal combustion engine used for such a vehicle makes it possible to accomplish a satisfactory state of specific fuel consumption by setting a suitable operation condition and for this purpose it is necessary to continually set a suitable gear ratio or proper travel of an accelerator pedal . this problem has become a substantial problem in this energy conserving age . in this invention , a simple circuit is used to produce a gasoline economy running indicator . the electrical circuit is composed of a pulse-voltage converter and a voltage signal proportional to a travelling amount of an acceleration pedal with the diesel engine . the voltage signal is then compared with a voltage value corresponding to an acceleration pedal travelling amount by a comparator . in obedience to such warning indication , the driver can continuously control the engine such that the engine is operated within the area defined by the lines and , thus , the vehicle is permitted to repeat the accelerating operation approaching the minimum fuel consumption rate as closely as possible . in addition , it is easy to change an amount of travelling of the accelerator pedal by merely judging the illumination of one of the lamp 5 and the red lamp 6 , which is not required to have any new operating techniques in addition to a conventional technique . furthermore , since the present invention is constructed by a very simple circuit such that the output signal of the characteristic converter 2 is compared with an detected signal other than the detected signal input to the converter 2 , the unit cost of production is reduced . therefore , the mounting cost of the overall system is economical . the present invention also provides a novel fuel economy running indicators indicating whether an accelerator pedal travelling amount determining the power output state of the internal combustion engine and a gear ratio of speed change gears , which are selected from some operating parameters varied by a driver , is within the scope of an economy fuel consumption , which can be used to minimize the fuel cost in operation in consideration of a high vehicle speed as required on an express highway and a power enough to accelerate the vehicle , such as during the running in the town . in conclusion , according to the present invention it is possible to reduce the fuel consumption within the limits of possibility , effecting the high-speed running required of an automobile and the repetition of the accelerating and decelerating operations .

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CROSS REFERENCE TO RELATED APPLICATION This application is a continuation-in-part of prior copending application Ser. No. 531,907 filed Sept. 12, 1983, nowPatent. TECHNICAL FIELD The present invention relates generally to a closure for pressure vessels. More particularly, this invention concerns an improved closure assembly for use with high pressure feedwater heaters, like those utilized in power plants, which is particularly adapted to facilitate better sealing and easier disassembly after operation for servicing, etc. BACKGROUND OF THE INVENTION In power generating plants, heat transfer between the steam and water is accomplished by means of feedwater heaters. Such heaters typically operate under high pressures on the order of 1,000 to 5,000 PSI, and must be constructed to withstand such pressures and to permit access to the interior for inspection, cleaning and the like. Feedwater heaters generally comprise a cylindrical casing, a removable closure assembly, and a seal therebetween. The closure assembly is usually designed so that internal pressurization of the feedwater heater facilitates and maintains a proper seal with the casing. Metallic gaskets of various types have been used for this purpose, however, the designs of the closure assemblies of the prior art have tended to cause extreme deformation of the gaskets, shortening their service lives and making removal of the closure assemblies a difficult, time consuming task. Specialized equipment and techniques have been required to remove the closure assembly from the casing in some circumstances. It is thus desirable to design the closure assembly and seal to provide effective sealing when the feedwater heater is under pressurization, while enabling complete disassembly after operation without the difficulties which have characterized the prior art. Patent shows a fluid-tight closure apparatus representative of one prior art approach to this problem. In this device, the inside end of the closure wall is formed to receive a circular pressurizing ring which contacts a surrounding seal and packing ring to effect sealing engagement with the inside surface of the casing. This approach, however, requires specially formed parts and is still subject to some of the difficulties of the prior art. Since all of the compression force is transmitted through the gasket, a relatively large bearing area is required. This type of gasket is usually of one-piece construction, which is expensive and not readily available. Further, after compression, this gasket also tends to wedge between the adjacent parts making separation and removal difficult. SUMMARY OF INVENTION The present invention comprises an improved high pressure feedwater heater closure, and particularly an end assembly and seal therefor, which overcomes the foregoing and other difficulties associated with the prior art. In accordance with the invention, there is provided a closure assembly for a pressure vessel which incorporates unique design features and a special seal arrangement to effect a proper seal while permitting convenient disassembly without special tools or undue difficulty. In a first embodiment, the closure assembly comprises an end piece or channel cover which is slideably received in the open end of the casing and retained in place against outward axial movement by a split retaining ring. Provided between the end piece and retaining ring are an o-ring seal, seated in a groove chamfered in the outer edge of the end piece, and a compression ring which overlies the o-ring and a portion of the end piece such that only a predetermined portion of the compression force is directed through the o-ring seal. The compression force exerted by the end piece upon internal pressurization is transferred in part by the compression ring without undue deformation of the o-ring such that the closure assembly can be readily removed, as desired. In a second embodiment, the end piece or channel cover is retained in place in the casing by the split retaining ring and a holding ring. 1 and 2, there is shown a portion of a feedwater heater 10 of the type which is typically used in power generating plants. Feedwater heaters of this type can be about 25-60 feet long and 24-50 inches in diameter, capable of operation under pressures of 1,000-5,000 PSI, and are therefore of relatively massive construction. The feedwater heater 10 includes a cylindrical channel or casing 12 connected to one end of a shell not shown which has been omitted for purposes of clarity. The casing 12 includes an inlet 14 and an outlet 16 opening into its interior, which is divided into two subchambers by means of a lateral divider or partition 18 and a semi-circular cover 20 which is secured by bolts 22 to the partition and a semi-circumferential shoulder 24 secured to the interior of the casing. A number of tubes 26 are provided in the closed end of the casing 12. The tubes 26, of which there are typically many, can be U-tubes having one end connected to the subchamber adjacent the inlet 14 and the other end connected to the subchamber within partition 18 and cover 20 adjacent the outlet 16. A first fluid, such as feedwater, is circulated through the casing 12 and tubes 26, while a second fluid, such as steam, is circulated through the shell not shown of the feedwater heater 10 so as to effect heat transfer between the fluids. A removable closure assembly 28 incorporating a first embodiment of the invention is provided in the open end of the casing 12. As will be explained more fully hereinafter, the closure assembly 28 utilizes a unique seal arrangement which provides a good seal with the casing 12 when the feedwater heater 10 is operating under pressure, and which also facilitates complete removal of the closure assembly for servicing, maintenance and the like after shutdown of the feedwater heater. Although the closure assembly 28 herein is illustrated and described particularly for use with feedwater heaters, it will be understood that the closure assembly of the invention can be adapted for use in other types of pressure vessels wherein periodic, convenient access to the interior is desirable. The removable closure assembly 28 includes a cylindrical end piece or channel cover 30 which is dimensioned for slidable receipt in the open end of the casing 12. The channel cover 30 is of relatively thick, heavy construction, and pulling lugs 32 are provided on the outside end of the cover to facilitate pulling it out of the casing 12. A threaded lifting hole 34 is provided in the side of the channel cover 30 to facilitate lifting and handling. It will thus be apparent that the channel cover 30 defines one end wall of the chamber within casing 12. A split retaining ring 36 and a position ring 38 are provided for constraining the channel cover 30 against outward axial movement from the casing 12. The outside periphery of the split retaining ring 36 is stepped as shown for receipt by an inside circumferential groove 40 formed in the casing 12. The inside periphery of the retaining ring 36 is angled as shown so that the segments of the ring are wedged outwardly into the groove 40 upon insertion of the solid position ring 38. A threaded tie bolt 42 and nut 44 are provided for pulling together the channel cover 30 and position ring 38 to effect such wedging action. The bolt 42 is screwed at one end to the outside end of the channel cover 30, while the nut 44 is provided on the other end, which passes through a hole in a tie bar 46 bridging the position ring 38. 2 and 3, a seal comprising an o-ring 54 and compression ring 56 are provided between the channel cover 30 and split retaining ring 36. The o-ring 54 is positioned in a bevelled groove 58 formed by chamfering the edge of the outside end of the channel cover 30. The o-ring 54 can be of any suitable construction, in accordance with the fluids and operating pressures of the particular application. The o-rings 54-a and 54-b can be of copper or other suitable metal. O-rings suitable for use as o-ring 54 are commercially available from Helicoflex Company of Boonton, N.J., and from Advanced Products Company of North Haven, Conn., for example. In the feedwater heater 10 as shown, the o-ring 54 is preferably formed of copper or otherwise metallized. however, the o-ring could be formed of other materials in different applications. It will be noted that the compression ring 56 overlies the o-ring 54 and a portion of the channel cover 30. This comprises an important feature of the invention, together with location of the o-ring 54 in the bevel groove 58. Tightening the pull-up bolts 60, which extend through holes in the split retaining ring 36 and screw into the channel cover 30, preloads the o-ring 54 by compressing it inward in the bevel groove 58 and thus outward against casing 12, to form an initial seal between the closure assembly 28 and the casing. When the feedwater heater 10 is in operation, of course, the internal pressure on the inner end of the channel cover 30 actuates and thus maintains compression of the o-ring 54 in order to provide an effective seal between the closure assembly 28 and the casing 12. The compression ring 56 contacts the split retaining ring 36 and bears upon both the o-ring 54 and the channel cover 30 such that a portion of the compression force from the channel cover is transferred directly into the split retaining ring 36. The o-ring 54 is compressed in the bevel groove 58 sufficiently to form a good seal, but since only a predetermined portion of the compression force is directed through the o-ring, it does not become crushed or deformed to such an extent that it impedes removal of the channel cover when the closure assembly 28 is disassembled. This prior art seal arrangement incorporates a soft copper gasket 62 seated in a notch, together with a narrow compression ring 64. It will be noted that the compression ring 64 overlies only the soft copper gasket 62 such that, when the casing is pressurized, all of the compression force from the channel cover is transmitted to the split retaining ring via the gasket. This arrangement concentrates the forces through the gasket such that it deforms severely and flows partially into the annular space between the channel cover and casing, which in turn leads to extreme difficulties in removing the channel cover. The present invention comprises a significant improvement over this prior art approach. 5 and 6, there is shown a removeable closure assembly 66 incorporating a second embodiment of the invention. The closure assembly 66 includes several components which are substantially identical in construction and function to corresponding components of the closure assembly 28 of the first embodiment. 1 and 2, but have been differentiated by means of prime 39. notations. The primary distinction between the two embodiments comprises the fact that the closure assembly 66 incorporates a solid holding ring 68 over the end of the casing 1239.. The holding ring 68 overlies the end of the casing 1239., but does not bottom out on either the split retaining ring 3639. or the solid position ring 3839.. The thicknesses of the split retaining ring 3639. and the solid position ring 3839. are relatively less than those of the first embodiment so that the holding ring 68 does not rest on these parts. Threaded tie bolts 70 and nuts 72 are provided for pulling together the channel cover 3039., compression ring 5639., and split retaining ring 3639. in order to obtain even better sealing, particularly under cyclic operating conditions. The tie bolts 70 extend through clearance holes in the holding ring 68 and the split retaining ring 3639., and are screwed at their inner ends to the channel cover 3039. in similar fashion to the pull-up bolts 60 of the first embodiment. The closure assembly 28 of the first embodiment, however, incorporates pull-up bolts 60 secured between the channel cover 30 and the split retaining ring 36, whereas the closure assembly 66 of the second embodiment utilizes tie bolts 70 and nuts 72 secured between the channel cover 3039. and the holding ring 68. If desired, pull-up bolts can be substituted for tie bolts 70 and nuts 72 in some applications, and it will be understood that these connectors are considerably fully equivalent. In addition, the closure assembly 66 preferably includes a relatively wider compression ring 5639. having a smaller internal diameter than the compression ring 56 of the first embodiment, so as to provide more bearing area between the channel cover 3039. and the split retaining ring 3639.. for passage of the tie bolts 70. If desired, shims 74 can be added between the channel cover 3039. and casing 1239. for centering purposes. In all other respects, the closure assembly 66 functions substantially the same as the closure assembly 28. From the foregoing, it will thus be apparent that the present invention comprises an improved closure assembly for pressure vessels having several advantages over the prior art. In the prior art, all of the compression force was transmitted through the gasket in order to obtain a good seal, however, this caused severe deformation which later made it extremely difficult to complete disassembly of the closure assembly without special tools and techniques. In the present invention, a controlled portion of the compression force is transmitted through the o-ring seal, with the remainder being carried directly by the compression ring such that an effective seal is maintained without undue deformation of the o-ring. Other advantages will be evident to those skilled in the art. Although particular embodiments of the invention have been illustrated in the accompanying Drawings and described in the foregoing Detailed Description, it will be understood that the invention is not limited to the embodiments disclosed, but is intended to embrace any alternatives, equivalents, modifications andor rearrangements of elements falling within the scope of the invention as defined by the following claims.

Original abstract:

The specification discloses an improved removable closure assembly (28) for use in feedwater heater or other pressure vessels requiring periodic internal access thereto for service, etc. The closure assembly (28) includes an o-ring seal (54) seated in a groove (58) chamfered into the outer edge of one end of a cylindrical cover (30) disposed in the open end of the channel or casing (12) of the pressure vessel. A compression ring (56) is disposed between the cover (30) and a split retaining ring (36) which are drawn together by bolts (60). The compression ring (56) overlies the o-ring (54) and a portion of the cover (30) so that only a portion of the compression force is transmitted through the o-ring, whereby an effective seal is provided without undue deformation such that the closure assembly (28) can be readily disassembled and removed, if desired. In a second embodiment, the closure assembly (66) incorporates a holding ring (68) which is secured by the bolts (70, 72) to the cover (30) over the retaining ring (36&#39;) and compression ring (56&#39;).

Predicted:

the present invention comprises an improved closure assembly for pressure vessels which incorporates unique design features and a special seal arrangement to effect a proper seal while permitting convenient disassembly without special tools or undue difficulty . in a first embodiment , the closure assembly comprises an end piece or channel cover which is slideably received in the open end of the casing and retained in place against outward axial movement by a split retaining ring . provided between the end piece and retaining a ring are an o-ring seal , seated in a groove chamfered in the outer edge of the end piece , and a compression ring which overlies the o -ring and a portion of the endpiece such that only a predetermined portion of the compression force is transmitted through the o-r seal . the compression force exerted by the end piece upon internal pressurization is transferred in part by the compression ring without undue deformation of the oring such that the closure assembly can be readily removed as desired . in the second embodiment , a separate closure assembly incorporating a second embodiment of the invention incorporates a solid holding ring over the end of the caing which does not bottom out on either the split retaining or the solid position ring , whereas the closure assembly 66 includes several components which are substantially identical in construction and function to corresponding components of the closure assembly of the prior art . it will be understood that the present invention can be adapted for use in other types of pressure vessels wherein periodic , convenient access to the interior is desirable , and that the closure assemblies of the invention can be used in other pressure vessels , wherein special tools and techniques are required to remove the closure assembly from the casing . in addition , it will be appreciated that the present closure assembly of pressure vessels can be easily adapted for other types ofpressure vessels wherein a special closure assembly is desirable . the present invention also includes a unique closure assembly that utilizes a unique seal arrangement to provide an effective seal while enabling convenient dis - assembling after operation for servicing and the like after shutdown of the feedwater heater , which is particularly adapted to facilitate better sealing and easier dis - dissembly after operation for operating conditions like feedwater heaters .

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