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Post-kala-azar dermal leishmaniasis – an overview

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Summary

Post-kala-azar dermal leishmaniasis (PKDL) is a dermal sequela of visceral leishmaniasis (VL), reported mainly from two regions – Sudan in eastern Africa and the Indian subcontinent, with incidences of 50–60% and 5–10%, respectively. Importantly, patients with PKDL are considered as reservoirs of VL, linking its eradication to effective control of PKDL. The etiopathogenesis of PKDL is presumably due to an immunological assault on latent dermal parasites. Immunological markers include IL-10, whose expression in skin and plasma of Sudanese patients with VL predicted onset of PKDL. Cell-mediated immune responses, notably restoration of IFN-γ production by antigen-stimulated lymphocytes are well documented in Sudanese PKDL, but remain ambiguous in the Indian form; recently, antigen-specific IL-10-producing CD8+ lymphocytes have been implicated in pathogenesis. In Indian PKDL, upregulation of intralesional IFN-γ and TNF-α is counterbalanced by IL-10 and TGF-β together with downregulated IFN-γ R1. Although IL-10 curtails excessive IFN-γ-mediated reactivity and ensures parasite survival, its cellular source remains to be confirmed, with infiltrating regulatory T cells (Tregs) being a likely candidate. Future functional investigations on Tregs and their interaction with lesional effector lymphocytes would be indispensable for development of immunomodulatory therapies against *Leishmania* infection.

Early Reports

Post-kala-azar dermal leishmaniasis (PKDL), a dermatosis generally observed in patients with a previous history of kala-azar (KA) or visceral leishmaniasis (VL),[1] was first described in 1922 by the eminent Indian physician-scientist, Sir U. N. Brahmachari (1873–1946). At a meeting of the Asiatic Society of Bengal, he presented four cases with unique dermal involvement, all of whom had been successfully treated for kala-azar, time interval varying from 6 months to 5 years.[2] He advocated the term ''dermal leishmanoid'' to describe the condition, as Leishman-Donovan (LD) bodies were observed in lesional smears. Eventually, following further studies by Shortt and Brahmachari, Acton and Napier, Knowles and Das Gupta, and other workers at the Calcutta School of Tropical Medicine, the disease was renamed as post-kala-azar dermal leishmaniasis.[3]

Epidemiology of PKDL

Incidence of PKDL is confined mainly to two regions endemic to kala-azar – the Indian subcontinent and Sudan plus adjoining areas, although case reports have emanated from China, Iraq, and Iran.[4,5] Parasites responsible for VL and subsequently PKDL belong to the *L. donovani* complex that includes *L. donovani*, *L. infantum* and *L. chagasi*. However, *L. amazonensis* that generally causes cutaneous (CL) and mucocutaneous leishmaniasis (MCL) in South America, has been implicated in both VL and PKDL.[6] In the Indian subcontinent, studies on Sri Lankan CL have confirmed dual cutaneo-viscerotropism of *L. donovani*;[7,8] *L. donovani* has also been confirmed as the causal species in Indian patients with localized CL (LCL) in a northern state, far removed from the kala-azar endemic region.[9] PKDL so far has been reported to be caused primarily by *Leishmania donovani* both in India[10] and Sudan,[1] with only a few cases reportedly caused by *L. infantum* or *L. chagasi*.[10]

Considering the increasing global incidence of HIV-VL co-infection, PKDL has been reported as an immune reconstitution inflammatory syndrome (IRIS) in a few patients following administration of anti-retroviral therapy.[11] Typing of these isolates would help determine the role of parasite-specific factors in co-infection but such information is to date limited.

Patients With PKDL: Reservoirs of KALA-AZAR[1]

Unlike in Africa where speculation continues to be rife on whether transmission of kala-azar is anthroponotic or zoonotic, transmission in India is anthroponotic, with *Leishmania* parasites surviving and propagating intra-dermally particularly in the intervening periods between successive VL epidemics. Patients with PKDL have thus acquired epidemiological significance of such magnitude, that as few as 0.5% of PKDL patients during a VL epidemic can potentially succeed in making VL endemic.

The stoppage of insecticide spraying under the National Malaria Eradication programme in the late 1970s possibly led to a resurgence in the sandfly population, whose feeding on patients with PKDL possibly triggered subsequent epidemics of kala-azar in Bihar and West Bengal; in fact, during the interepidemic period, PKDL cases exceeded VL cases, corroborating that patients with PKDL indeed serve as disease reservoirs. However, during an epidemic of VL, a greater number of PKDL cases in a calendar year coincided with a surge in VL cases, corroborating that resurgence of VL is intimately linked to the presence of a pool of patients with PKDL.

Entomological studies have provided direct evidence that *Phlebotomus argentipes*, the proposed vector of VL in India, when allowed to feed on PKDL patients, get infected and develop promastigotes in their midgut, making them capable of transmitting the parasite. Indirect evidence has been accrued from a study wherein isolates from patients with PKDL showed antimony unresponsiveness,[12,13] accounting for the increased clinical resistance to antimony.[14,15] Between the two probable reservoirs of VL, namely patients with VL and patients with PKDL, the exposed skin lesions of the latter are easily accessible areas for the sandfly vector to ingest *Leishmania* parasites.

PKDL, A Drug-Dependent Manifestation?

Thakur *et al.*[16] reported a distinct reduction in cases of PKDL despite an increased incidence of VL, attributing this to the introduction of Amphotericin B as a frontline drug against kala-azar. Saha *et al.*[17] demonstrated that treatment with sodium antimony gluconate (SAG) and amphotericin B had markedly different effects on levels of IL-10 and TGF-β in patients with PKDL and VL, suggesting that PKDL was more likely to follow in patients with VL who had been treated with SAG. A recent report has also documented PKDL following miltefosine treatment in two Indian cases of VL.[18] As a result of the paucity of relevant epidemiological information on PKDL, more so in India than in Sudan, prospective clinical trials over a period of 2–3 years have been suggested for validating the hypothesis that PKDL emerges following antimonial treatment of VL.[10] However, the low incidence of PKDL in India is a significant hurdle and must be taken into account while conducting prospective studies.

Clinical Features[1,4]

The Indian and African forms of PKDL present with striking similarities in that the disease begins with small measles-like lesions (hypopigmented macules, papules or nodules) usually appearing on the face and gradually increasing in size (Figs 1 and 2). Eventually, the lesions spread to the torso, extremities, neck, and back. The multiple lesions can coalesce to form larger lesions resulting in gross enlargement of the nose and lips (Fig. 2). PKDL is often confused with leprosy, secondary syphilis and sarcoidosis. Occasionally, there is secondary involvement of nasal mucosa, hard and soft palate, oropharynx, larynx or eye lids and cornea, leading to blindness.

In Sudanese PKDL, nodular or papular lesions have the highest incidence (51%) followed by maculopapular (23%), micropapular (17%) and macular (9%) lesions. In the Indian clinical setting, erythema and induration on the butterfly area of the face with variable photosensitivity, symmetrically dispersed hypopigmented macules that may sometimes coalesce (Fig. 1), and a polymorphic assortment of papules, nodules, macules, and/or plaques are typically observed (Fig. 2). The percentage distribution of macular and polymorphic lesions reported by Thakur *et al.*[19] was 23 and 45%, respectively (nodular and

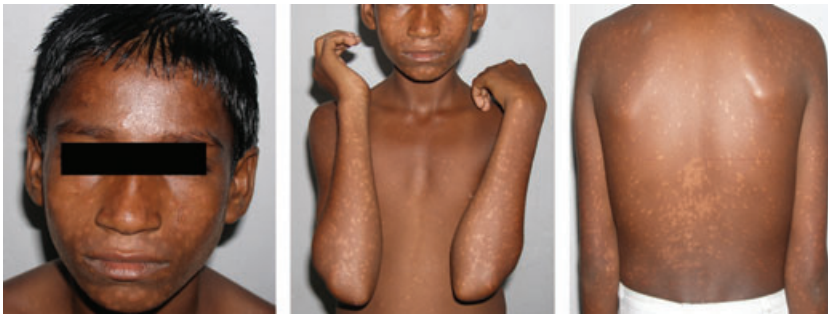

Figure 1 Clinical photographs of an Indian patient with PKDL presenting with exclusively macular lesions (Written consent was obtained prior to photography)

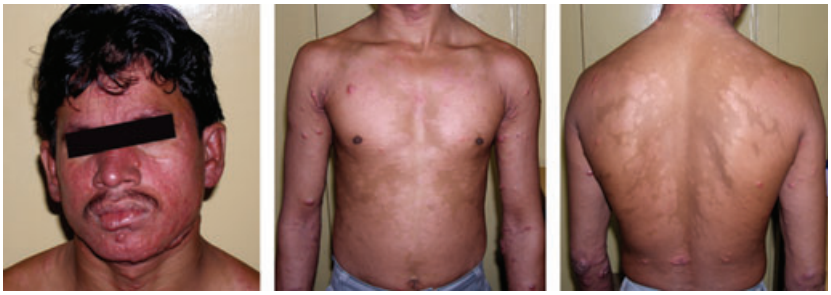

Figure 2 Clinical photographs of an Indian patient with polymorphic PKDL presenting with nodules, papules and macules (Written consent was obtained prior to photography)

papular lesions accounted for 32%); Saha *et al.*[20] recorded 15% macular and 85% polymorphic cases, respectively, which was in agreement with our study (69% polymorphic and 31% macular).[21] Regardless of variations in clinical profiles from regions where PKDL is reported, it is generally assumed that nodular, macular, and maculopapular lesions predominate and are construed as hallmarks of PKDL.

Histopathology[1,22]

In a detailed histological and immunohistochemical investigation on dermal tissue from Sudanese PKDL patients, the epidermis exhibited several changes in different combinations; however, a consistent feature was dermal infiltration by lymphocytes and macrophages. Very few plasma cells were seen, if at all, which was in contrast to lesions of cutaneous leishmaniasis caused by *Leishmania major*. The majority of cells were $CD3^+$ T lymphocytes, with $CD4^+$ helper lymphocytes predominating over $CD8^+$ cytotoxic lymphocytes. Acute degeneration in basal keratinocytes was observed, which were also positive for HLA-DR, ICAM-1 and *Leishmania* antigen as determined by specific antibody-binding. This degeneration was attributed to the cytotoxic action of infiltrating lymphocytes upon interaction with *Leishmania*-expressing epidermal cells. Melanocytes with whom lymphocytes were in contact also appeared to be damaged by infiltrating cells, accounting for the observed depigmentation. Hyperplasia of the B-and T-cell zones was evident in regional lymph nodes. In PKDL lesions, a neuritis involving small cutaneous nerves has also been documented thus having the disturbing potential of confounding diagnosis, owing to similarity with neuritis typically observed in leprosy. Histopathological reports documenting presence of parasites in lesional biopsies differed with type of rash and duration of disease.

In Indian PKDL, the main histopathological features include a diffuse dermal infiltrate of lymphocytes, plasma cells, and macrophages (Fig. 3). The epidermis shows distinct signs of atrophy with prominent follicular plugging often leading to the appearance of epidermal cysts, a feature that, together with absence of nerve involvement, helps distinguish it from leprosy. To date, there is only one solitary report of neuritis in Indian PKDL. Parasites have been documented in approximately 90% of lesional sections, more so in nodular rather than papular or macular lesions. However, it is generally observed that sparse or no Leishman–Donovan (LD) bodies constitute a histopathological feature of Indian PKDL. This diagnostic bottleneck is attributed to the difficulty in recognizing the scarce parasites by routine histopathological methods. Additionally, the empirical use of anti-leprosy drugs can also temporarily decrease the parasite load. Newly developed immunohistochemical methods have facilitated

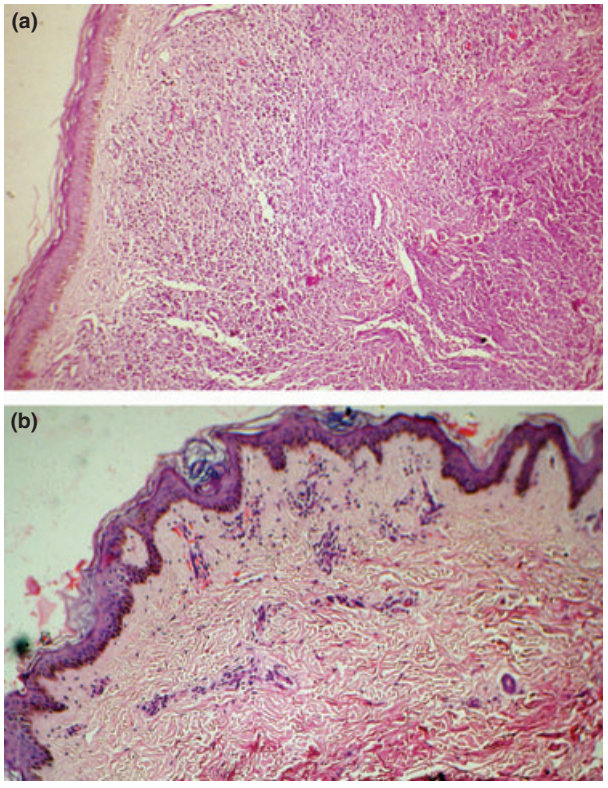

Figure 3 Histologic profiles of (a) nodular lesion and (b) macular lesion of Indian PKDL (hematoxylin and eosin ×10)

easier identification of *L. donovani* parasites, located mainly in the superficial dermis, which is also associated with the maximum degree of inflammation.

Etiopathogenesis[1,10,22]

As PKDL develops after cure in certain patients with VL, it is believed that in these patients, a few parasites (for reasons not established) evade destruction and possess the potential to afflict the skin. PKDL following inadequate therapy for VL in Sudan has been documented. PKDL could also be caused by reinfection of an individual rendered immune to visceral infection following treatment for VL or even by reactivation of latent parasites. It has also been argued that treatment and subsequent immune activation forces the parasites to seek refuge within the dermis possibly accounting for why PKDL appears following completion of therapy. However, this paradigm does not hold for the Sudanese variant where PKDL occurs concurrently with VL. When investigated at the molecular level, clinical isolates from Sudanese and Indian patients with PKDL have consistently been typed as *L. donovani* and to date no unique strain responsible for PKDL has been identified. Appropriately, PKDL is not considered a parasite-determined pathology, unlike the other leishmaniases.

It has been postulated that although the causative parasites isolated from both KA and PKDL patients are similar, adaptation to different tissue environments predicates involvement of genetic determinants; in that context, polymorphism observed in a specific genetic locus among strains (from VL and PKDL) could explain the altered tissue tropism. Microarray analyses of PKDL isolates have shown an upregulated expression of several surface proteins, e.g. the metalloprotease glycoprotein, gp63 and the promastigote-specific antigen, gp46. These proteins have been suggested to account for the parasites' ability to migrate through the extracellular matrix by digesting some of its components as also by virtue of their ability to resist complement mediated lysis. These structural modifications tend to confer survival benefits, enabling the parasites to survive the onslaught of anti-leishmanial drugs administered during treatment for VL, ultimately causing PKDL.

Differences between Sudanese and Indian PKDL[1,22]

Irrespective of similarities in presentation, striking differences exist between Sudanese and Indian PKDL. The disease occurs in 50–60% of Sudanese VL patients whereas in India, the incidence is substantially lower, ranging from 5 to 10%. In Africa, the time interval between cure of VL and onset of PKDL is 0–13 months and the term "Para Kala-azar dermal leishmaniasis" has often been used to describe patients who develop PKDL concomitantly even while receiving treatment for VL. These short time intervals have translated into successful longitudinal studies on Sudanese VL patients who later developed PKDL. However, with regard to Indian PKDL, longitudinal studies although desirable, are logistically impossible owing to the prolonged time interval between cure of VL and onset of PKDL which ranges from anywhere between 1 and 7 years and sometimes extends even up to 20–30 years. Furthermore, the lower incidence of PKDL in India complicates validation of prognostic biomarkers.[23]

Diagnosis[1,23]

A diagnosis of PKDL is typically based on a history of VL, distribution and appearance of the lesions, and by parasitological confirmation when diagnosis is doubtful. Indian studies with skin slit smears from PKDL lesions have shown a greater probability of demonstrating amastigotes in nodular lesions (67–100%) as compared to papular (36–69%) and macular lesions (7–33%). Cultures, which theoretically would give higher parasite yield as compared to smears, have a tendency to get contaminated. In our experience with transformation of parasites from PKDL lesions, only nodular tissue yielded viable promastigotes. Of a total of 35 samples, 8 (all were from nodular lesions) transformed successfully and only 3 could be sustained in culture, which subsequently were tested for their chemosensitivity profiles.[13]

Diagnosis using monoclonal antibodies and PCR-based assays is more definitive but is handicapped by inherent limitations of price and infrastructure, restricting their use in the field setting. Validated serological tests for VL such as the direct agglutination test (DAT) and ELISA do not hold much value for serodiagnosis of PKDL, especially in endemic areas as antibodies to *L. donovani* can persist for a long time even after cure of VL, confounding interpretation of a positive test result in a suspected case of PKDL with a documented history of VL; consequently, no specific serodiagnostic test has ever been validated for PKDL. Indian patients with PKDL and VL had comparable rK39 IgG titres and the same trend was reported from Bangladesh with regard to DAT titres. In Sudan, the rK39 strip test had similar specificity and sensitivity in patients who had been cured of VL but did not develop PKDL vis-à-vis patients who developed PKDL. This is possibly due to the short time-interval between cure of VL and onset of PKDL. Among other serological tests, a competitive ELISA using *L. donovani*-specific monoclonal antibody showed good results in all seven Indian PKDL patients tested while the rK39 strip test detected 91% of

Indian PKDL cases with absolute specificity. In our study, patients with PKDL tested positive for rK39 antibodies (immunochromatographic strip test) along with high levels of anti-leishmanial antibodies on an indirect ELISA; their absorbances were however significantly lower than patients with VL.[21] If the time interval between occurrence of VL and appearance of lesions (attributable to PKDL) is less than 1 year, there would be a diagnostic dilemma as the rK39 immunochromatographic test being an antibody-based diagnostic test, would be positive in both cases. Therefore, in such cases, it is recommended that the lesional smear be examined together with culture test and PCR to confirm the diagnosis of PKDL.

Immunopathogenesis of PKDL

Although PKDL was described in India decades ago as a clinical sequela manifesting within 6 months–5 years of cure of kala-azar,[2] the immune pathways that underpin the pathogenesis of PKDL continue to remain a subject of considerable debate.[1,22] What has been proven beyond doubt is that immunological features of PKDL (the sequela with only dermal involvement) differs from VL (the systemic ''forerunner'') in several aspects. The immunopathology of VL is characterized by a pronounced suppression of cell-mediated immunity (CMI), particularly in response to the *Leishmania* parasite, together with an upregulated humoral response (hypergammaglobulinemia and formation of immune complexes). Successful treatment restores CMI and confers resistance to reinfection.[24] PKDL, on the other hand, develops in a small proportion of cured VL patients, presumably as a result of developing immune responses to parasites present in the skin.[1,22]

Cell-mediated immune (CMI) responses in PKDL

In Sudanese PKDL, lymphoproliferative responses to *Leishmania* antigen were consistently associated with IFN-γ production in all patients, with co-production of IL-10 in 20%.[25] In a longitudinal study involving Sudanese patients with VL who went on to acquire PKDL, raised IL-10 levels in plasma and keratinocytes was evident, highlighting the importance of IL-10 as a predictor of disease.[26] In a subsequent study, the same group also showed a strong association between onset of PKDL lesions and *Leishmania*-specific activity of peripheral blood mononuclear cells (PBMC) from patients with VL, as evident in the stronger lymphoproliferative responses.[27]

Indian studies have however differed on the status of CMI responses in PKDL possibly due to smaller study populations. Haldar *et al.*,[28] reported an increased antigen-specific CMI, that was more evident in newly acquired PKDL (5/6 patients, time intervals varied from a few months to a year) than chronic PKDL (3/6 patients whose time intervals ranged from 8 to 30 years). Interestingly, in a study by Neogy *et al.*,[29] antigen-specific responses were non-detectable in any of the 10 patients studied, while responses to phytohemagglutinin remained intact. In contrast, patients with VL were deficient with regard to both antigen-specific and generalized CMI responses but with treatment, specific and non-specific CMI responses were restored.[29] Saha *et al.*[17] analyzed antigen-specific proliferative responses in 11 patients with PKDL and showed the response to be inversely related to severity of pathology; interestingly, severity was directly linked to higher IL-10 and TGF-β secretion by antigen-stimulated PBMC.[17] Studies by our group on Indian patients with PKDL demonstrated strong CMI responses in circulating PHA-stimulated lymphocytes in terms of intracellular IFN-γ and IL-2 production while antigen-specific CMI was non-detectable and showed high levels of intracellular IL-10 in antigen-primed $CD3^+CD8^+$ lymphocytes.[21]

The presence of circulating pro- and anti-inflammatory cytokines is a well-documented feature of VL.[30,31] In Indian PKDL, serum levels of IFN-γ, IL-10 and IL-6 as measured by cytometric bead array were lower than patients with VL and similar to healthy controls, emphasizing that the immune response in PKDL is localized.[32] However, our investigations showed elevated serum levels of IFN-γ and IL-10 selectively in polymorphic PKDL, comparable to patients with VL, while levels of IFN-γ and IL-10 were much lower in macular PKDL, suggesting that differences in systemic cytokine profiles could account for the observed variations in disease pathology.[21]

Lesional immunology

Most Sudanese patients with PKDL have a lesional infiltrate rich in $CD3^+$ T cells, with a preponderance of $CD4^+$ over $CD8^+$ cells.[25] Further, the presence of macrophages in conjunction with expression of IL-10, IFN-γ and IL-4 was observed in most lesions from Sudanese patients, suggesting a balance is maintained between intralesional cytokines, which probably influences disease outcome.[25]

In Indian PKDL, one of the first studies on the lesional profile[33] highlighted the preponderance of $CD8^+$ over $CD4^+$ T cells within both dermal lesions and draining lymph nodes; this was more prominent in patients presenting with advanced nodular lesions. A similar predominance of $CD8^+$ over $CD4^+$ lymphocytes in both hypopigmented, macular and nodular/ plaque lesional tissue was reported from Indian patients with PKDL.[34]

On a molecular level, studies on the cytokine expression profiles within the localized milieu have established IFN-γ, IL-6, TNF-α, IFN-γR1 (receptor for IFN-γ) along

with IL-10 as being the primary immunodeterminants of disease pathology in Indian PKDL.[35] In the same study, the authors suggest that the raised mRNA levels of IL-10 and TGF-β indicates a role for these cytokines in disease pathogenesis, via derailment of Th1 responses. As a follow-up to that study, analyses of TNF-α receptor expression (TNFR1 and TNFR2) showed downregulated TNFR1 transcripts in both PKDL and VL.[36] Taken in conjunction with the previously reported high TNF-α expression, this impaired expression of the TNFR1 gene was therefore proposed to account for the impeded Th1 response in PKDL. The group also analyzed the status of matrix metalloproteinases, known to be induced by TNF-α and validated important roles for tissue inhibitors of matrix metalloproteinases (TIMPs), in the pathogenesis of PKDL.[36]

A recent investigation by our group, while corroborating previous reports of high intralesional co-expression of IFN-γ and IL-10 in Indian PKDL,[35] showed a significant reduction in mRNA levels of both cytokines following treatment in all 12 patients studied on an individual basis.[37] This data points to the involvement of mutually counteracting cytokines in disease pathogenesis, with IFN-γ acting against skin-resident parasites, while IL-10 attempts to curtail an excessive and potentially host-reactive IFN-γ-driven response (Fig. 4).

In murine leishmaniasis, analysis of the polymorphic gene SLC11A1, which influences both innate and adaptive immune responses, demonstrated that T cells are indispensable for maintenance of low parasite loads, facilitating chronic infection. In Sudanese VL, susceptibility has been linked to a polymorphism in the IL-4 gene while onset of PKDL was shown to be under the influence of an IFNGR1 polymorphism.[38] However, given the complex structure of cytokine signaling pathways, it is possible that disease severity and progression is determined by a summation of polymorphisms for different cytokines and their receptors.[38]

As regulatory T cells (Tregs) are potential sources of IL-10 particularly in chronic infection-specific milieus,[39] our group examined their presence in lesions from patients with PKDL and detected a significant degree of accumulation of Foxp3+ Tregs, concomitant with increased Foxp3 mRNA expression;[37] importantly, both Foxp3 mRNA levels and proportions of accumulating Tregs were responsive to treatment.

Is there a role for regulatory T cells and IL-10?

During any infection, immune regulation mostly follows as an aftermath of unchecked host responses to the pathogen with a view to protecting the host from immune-mediated damage.[39] Regulatory responses are mediated by cytokines, such as IL-10 and TGF-β, produced by

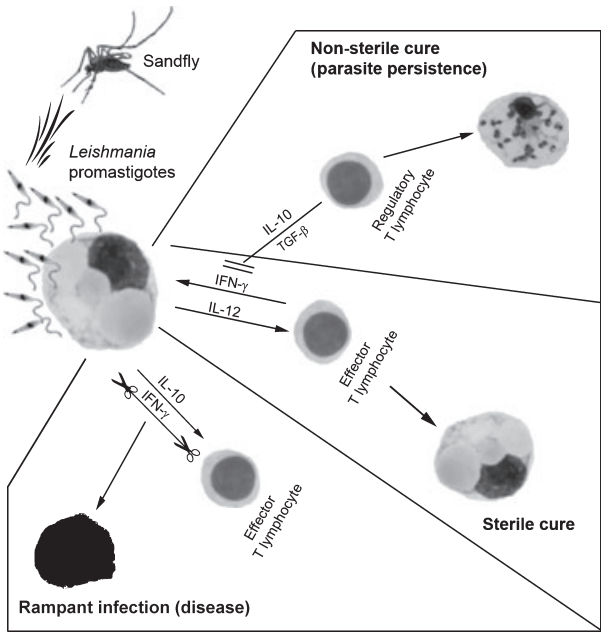

Figure 4 Schematic illustration of cell-mediated immune responses following human infection with *Leishmania* parasites introduced by the bite of the sandfly, leading to one of three possible endpoints: disease progression, sterile cure or parasite persistence, the last of which most likely explains the immunopathogenesis of PKDL. ✂ and ⊣ indicate curtailment of cytokine secretion

innate immune cells or indirectly via regulatory cells. $CD4^+$ regulatory T cells (Tregs) that represent 5–10% of the peripheral $CD4^+$ T cell population are characterized by expression of the transcription factor Foxp3 and are involved not only in maintenance of immune homeostasis but also in the regulation of immunity to infection.[40]

In experimental murine leishmaniasis, natural Tregs recognizing parasite-derived antigens accumulate at the site of infection.[41] A similar regulatory response has also been documented in humans with reports of Treg accumulation in lesions of CL caused by *Leishmania braziliensis* and their resultant downregulation of effector T cell responses.[42] In fact, in localized CL caused by *L. guyanensis*, unresponsiveness to treatment correlated strongly with high lesional Foxp3 expression, indicating a possible impairment of local immune responses by accumulating Tregs.[43]

IL-10, as a key immunoregulatory cytokine in chronic infections, is known to inhibit anti-parasitic immune responses experimentally[44] and also, in clinical leishmaniasis.[45] *L. major*-infected murine macrophages activated by immunoglobulin-containing immune complexes have been reported to secrete high levels of IL-10, which increases susceptibility to infection (Fig. 4).[46]

Both IL-10 and TGF-β have been associated with activation / differentiation of T regulatory (T_{reg}) cells.[47] In a murine model of *L. major* infection, disease chronicity was associated with IL-10 producing T_{reg} cells.[48] However, a role for natural Tregs in clinical VL was downplayed in a study that showed IL-10 expression by splenic T lymphocytes, that were notably not of the $CD4^+CD25^+Foxp3^+$ Treg phenotype.[49]

Unlike VL, PKDL is not a systemic disease and exclusively affects the skin. In Indian PKDL, severity of pathology appears to be dictated by the higher antigen-specific IL-10 and TGF-β production from circulating lymphocytes.[17] Ansari *et al.* demonstrated raised IL-10 expression in lesional tissue of Indian patients but the cellular source(s) were not identified.[35] Our study detected a significantly increased subpopulation of IL-10 expressing antigen-specific lymphocytes in peripheral blood of patients with PKDL and we speculated that these could be a subset of inducible regulatory T cells (Tregs; Fig. 4).[21] However, what still needs to be elucidated at the lesional site is the proportion of natural Treg cells vs. Treg cells with parasite-derived antigen.

In a subsequent study,[37] we have demonstrated an increase in $Foxp3^+$ Tregs within lesional tissue in support of our earlier hypothesis. As Tregs expressing both IFN-γ and IL-10 have been detected in cured patients with VL and as IL-10 is copiously expressed with concomitant downregulation of IFNGR1 in the dermal lesions of patients with PKDL, it has been proposed that the IL-10 rich immune milieu promotes recrudescence of *Leishmania* parasites in the skin.[38]

In addition to cytokines and transcription factors, chemokines or chemotactic cytokines secreted by immune cells are equally important in initiating the host immune response by directing cellular migration to the site(s) of infection. In patients with localized CL and nonhealing DCL, the chemokines CCL2 and MIP-1α were implicated in lesional macrophage activation. Further, the synergistic stimulation of macrophages by CCL2 and IFN-γ led to parasite elimination.[50 and refs therein]

Chemotherapeutic Responses[1]

In Sudanese PKDL, spontaneous healing occurs frequently. With no reports yet of antimony resistance in Sudan, sodium stibogluconate (SSG, pentavalent antimony) is used to treat cases wherein lesions have persisted for a year. In these cases, when 20 mg/kg SSG per day for 30 d does not yield satisfactory results, treatment can be extended by another 1–2 months, or alternative drug choices considered. As spontaneous healing is not a feature of Indian PKDL, therapy includes the frontline antileishmanial agent, sodium antimony gluconate (SAG) at 20 mg/kg per day for 4 months, cure rates varying from 64 to 92%. However, in view of the growing problem of antimony refractoriness in India, alternative therapeutic agents have been screened against PKDL. While Pentamidine was reportedly effective with a cure rate of 93% but had serious adverse effects, Ketoconazole was found to work better at a lower dose in combination with SAG. Administration of the anti-leprosy drug, Rifampicin, helped in a solitary case of PKDL who had not responded to SAG while allopurinol was effective in two patients after 20–24 months.

Amphotericin B has emerged as a capable frontline drug against VL, both safe and effective when used in management of SAG-unresponsive VL patients in India. In Indian patients with PKDL, amphotericin B (100% after 120 d) had a better cure rate as compared to SAG (64% after 400 d). In a recent case report, two miltefosine-treated VL patients who subsequently acquired PKDL, were administered amphotericin B; nodular lesions were resolved with one course of treatment but macular lesions required multiple courses.[18] The limitation with amphotericin B therapy is the expense and associated nephrotoxicity but the latter can be addressed by the administration of its safer but prohibitively expensive liposomal formulation. In a Sudanese study on PKDL patients who were administered liposomal amphotericin B (i.v. 2.5 mg/kg/d for 20 d), lesions were resolved fully in 83% of the patients with no adverse effects.[51] Miltefosine or hexadecylphosphocholine, an alkylphosphocholine analogue, can be administered orally and showed good efficacy against experimental *Leishmania*-infected mice.[52] Interestingly, miltefosine was developed as a novel drug candidate against breast cancer but because of severe gastrointestinal toxicity, was only approved as a topical formulation for dermal metastases.[53] Trials on Indian patients with VL have shown it to be a better alternative to existing parenterally administered drugs.[54] However, use of miltefosine for treating Indian PKDL has been limited, evident in just two case reports from India. The first patient, who did not respond to a 2 month regimen, was treated for an additional month and was fully cured[55] while the other received treatment for approximately 2 months, and showed complete resolution by 6 months.[56]

Immunochemotherapeutic modalities against PKDL, although desirable, are in need of optimization. In the only study so far on safety, immunogenicity and effectiveness of an immunochemotherapy regimen (alum-precipitated autoclaved *Leishmania major* vaccine + Bacille Calmette-Guerin and sodium stibogluconate) against Sudanese PKDL, a high cure rate (87% by day 60) was observed and importantly, minimal adverse events were recorded.[57]

Conclusions

Post-kala-azar dermal leishmaniasis (PKDL) is a poorly understood sequela to visceral leishmaniasis (VL) or kala-azar caused by *Leishmania donovani*, remaining confined to the Indian subcontinent and Sudan plus neighboring areas in Eastern Africa. Divergence between the two clinico-geographical forms vis-à-vis clinical features has prevented extrapolation of information from the more prevalent and widely researched Sudanese variant. Importantly, transmission of VL in India being anthroponotic, PKDL patients have been proposed as parasite reservoirs especially during interepidemic periods. Eradication of VL, particularly in India, is therefore contingent on our success in containing PKDL, which to date is limited by our poor understanding of its immunopathogenesis. Attempts have been made to dissect specific CMI responses with a view to ascribe roles in immunopathogenesis to effector and regulatory lymphocyte subsets and their secreted cytokines, both at the periphery and within the lesions. Future functional approaches could aim at deciphering the interactions between effector and regulatory cells in the local/peripheral milieu and yield potential targets for immunomodulatory agents against PKDL with the broader agenda of eradication of Leishmaniasis. Future investigations could also help in establishing our hypothesized role for lesional regulatory cells in promotion of parasite persistence and in the pathogenesis of disease progression, thereby making PKDL the reservoir of disease transmission, at least in India.

Questions

1. Post-kala-azar dermal leishmaniasis (PKDL) follows visceral leishmanisis (VL) in a few patients after successful treatment probably due to:
   a. *de novo* infection of the skin by Phlebotomine sandflies.
   b. Immune responses against latent parasites in the skin.
   c. Migration of parasites from infected organs in VL (liver, spleen, lymph nodes) to the skin.
   d. 'b' and/or 'c'.

2. Relevant immunological information of patients with PKDL vis-à-vis patients with VL has been easily available from Sudanese cases than from India. Which of the following best explains this discrepancy?
   a. PKDL often occurs concomitantly with VL in India while there is an indeterminate interval in Sudan.
   b. Sudanese cases are difficult to cure while Indian cases are easily cured.
   c. Sudanese cases often heal spontaneously but Indian cases require prolonged treatment.
   d. Long, often indeterminate intervals in India render longitudinal studies difficult to perform while they are easily performed in Sudan.

3. Which of these best describes the clinico-parasitological features of PKDL?
   a. Nodules with sparse parasites, macules with abundant parasites
   b. Papules with abundant parasites, macules with scanty parasites.
   c. Macules with abundant parasites, papules with scanty parasites.
   d. Nodules with abundant parasites, macules with scanty parasites.

4. Which of these symptoms often confounds the diagnosis of PKDL?
   a. Aggravated hypoesthesia.
   b. Coalescing lesions on the face and ears leading to a ''leonine facies'' appearance.
   c. Scattered hypopigmented lesions.
   d. All of the above.

5. The rK39 immunochromatographic strip test uses a strip coated with a recombinant kinesin peptide. What does the test detect?
   a. Peptide-reactive antigens in serum.
   b. Peptide-analogous antigens in serum.
   c. Peptide-specific antibodies in serum.
   d. 'b' and 'c'.

6. Serodiagnostic tests do not hold much value in PKDL as:
   a. Anti-leishmanial IgG generated during VL can persist in PKDL.
   b. Anti-leishmanial IgG are non-detectable.
   c. Anti-leishmanial IgM persist.
   d. None of the above.

7. Parasite-specific CMI observed in Sudanese patients with PKDL is deficient in a majority of Indian patients. Which of these probably explains the dichotomy?
   a. Long interval following cure of VL in India ensures depletion of antigen-reactive T lymphocytes from peripheral pools.
   b. Antigen-specific T lymphocytes are necessarily more responsive in Sudanese cases.
   c. Antigenic anergy is a feature of Indian PKDL.
   d. All of the above.

8. How is production of IL-10 by antigen-specific peripheral lymphocytes in PKDL associated with disease severity or even pathogenesis?
   a. Increased expression of IL-10 is parasiticidal and facilitates cure.
   b. IL-10, being a potent anti-inflammatory cytokine, promotes parasite survival and strongly correlates with disease severity.
   c. IL-10 drives onset of disease but then subsides and is negatively correlated with pathology.
   d. None of these.

9. Histopathologically, which of these features would explain the depigmentation observed in PKDL?
   a. Melanocytes are swamped by foamy macrophages.
   b. Melanocytes are greatly reduced in number in the hypopigmented lesions
   c. Melanocytes suffer structural damage after coming in contact with infiltrating lymphocytes.
   d. Melanocytes are functionally impaired in presence of *Leishmania* parasites.

10. This alkylphosphocholine analog was originally developed as a chemotherapeutic agent against breast cancer but because of adverse effects on the gastrointestinal tract, was later approved solely for dermal metastases. Trials have proved its efficacy against Indian visceral leishmaniasis and further trials are needed to determine its activity against PKDL. The drug being talked about is:
    a. Pentamidine.
    b. Ketoconazole.
    c. Paramomycin.
    d. Miltefosine.

Answers

1. d.
2. d.
3. d.
4. b.
5. c.
6. a.
7. a.
8. b.
9. c.
10. d.

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