CHAPTER VII

IRON HOMEOSTASIS IN PKDL

Introduction

Iron, an essential nutrient for living cells has diverse activities range from being a cofactor of metabolic enzymes, oxygen transport and for immune surveillance [Weiss, 2002]. Therefore, when a pathogen enters our body, the host and pathogen compete for the iron pool which then becomes a critical factor that influences disease progression. It has been known that iron homeostasis influences immunity and vice versa as Th1-Th2 cytokines reciprocally regulate the expression of genes involved in iron uptake and metabolism. Conversely, changes in iron concentration can influence the immune response against many pathogens [Theurl et al. 2005] as iron deficiency can lower the incidence of infectious diseases irrespective of the pathogen by causing a powerful Th1 immune response while increased accumulation of iron increases disease susceptibility [Reviewed in Vega and Korbi, 2006].

Monocytes/macrophages employ multiple strategies by which they can acquire iron, including (a) the transferrin-transferrin receptor (TfR) mediated iron uptake, (b) transmembrane uptake of ferrous as also ferric ion, (c) iron acquisition via lactoferrin receptors, (d) ferritin receptors and/or (e) erythrophagocytosis [Reviewed in Weiss G, 2002]. In addition, macrophages also acquire iron via phagocytosis of the haemoglobin-haptoglobin (Hb-Hp) complexes. CD163 being the receptor responsible for this endocytic process and is a member of the scavenger receptor family [Reviewed in Theurl et al. 2005].

Since development of infection is influenced by the availability of iron, bacteria and parasites have evolved diverse mechanisms to acquire iron from their immediate environment. In this regard, *Leishmania* is no exception and meticulously acquires iron from host macrophages by various approaches. To meet its requirement of iron, *Leishmania* enhances expression of iron acquisition transporter proteins on its membrane which includes (a) LIT1 (a ZIP family of transporters) and (b) a heme transporter [Reviewed in Huynh and Andrews 2008]. Additionally, *L. donovani* also activates host iron sensor proteins (IRP1 and IRP2), which via enhanced intracellular production of transferrin along with its receptor ensures further uptake of iron [Das et al. 2009]. Other sources include heme, lactoferrin, haemoglobin etc. present in the endosomal compartment of macrophages [Reviewed in Huynh and Andrews et al. 2008].

Iron in mammalian cells is primarily present in an oxidized ferric state (Fe^{+3}), which being poorly soluble cannot be taken up by the parasite. However, parasites possess a NADPH dependent Fe reductase that converts Fe^{+3} to Fe^{+2} which then helps uptake via an iron transporter LIT1/LIT2 [Reviewed in Huynh and Andrews et al. 2008]. The LIT1 protein is expressed only in amastigotes, suggesting its regulation by the macrophage environment [Marquis and Gros 2007].

The macrophage is the most important cellular player at the interface between iron and immunity as the availability of iron is necessary to produce the highly toxic microbicidal

hydroxyl radicals. At the same time, macrophages are major storage sites of iron under inflammatory conditions [Reviewed in Recalcati et al. 2012]. Our understanding of how macrophages control iron metabolism in infection and inflammation is even more complex owing to their functional diversity. Depending on their phenotype, M1 (classically activated) or M2 (alternatively activated) macrophages handle iron differently as the former has iron retaining capacity whereas M2 macrophages are known to actively export iron [Corna et al. 2010, Reviewed in Cairo et al. 2011]. This strategy of sequestering iron into macrophages helps to starve circulating microbes that would then be efficiently destroyed by activated M1 macrophages. However, in case of an intracellular pathogen, their survival is influenced by the iron withholding property of M1 macrophages. These M1 macrophages have a feedback inhibition mechanism by which following iron loading an inhibition of IFN-γ mediated pathways leads to decreased formation of pro-inflammatory cytokine TNF-α, expression of MHC class II antigens, formation of neopterin (a degradation of product of Guanosine triphosphate or GTP) and finally Tryptophan degradation via IFN-γ mediated induction of indoleamine-2,3 dioxygenase (IDO) expression which ultimately leads to these M1 macrophages being repolarized to either resting or a M2 macrophage phenotype [Reviewed in Vega and Corbi 2006].

There are important differences in iron handling properties of M1 and M2 macrophages. The M2 macrophages have a large labile iron pool (LIP) whereas M1 macrophages have a limited iron pool and uptake capacity [Corna et al. 2010]. The lowered LIP in IFN-γ/LPS stimulated M1 macrophages occurs due to the increased levels of NO which then causes upregulation of iron sensing protein (IRP) along with downregulation of ferritin translation culminating in decreased iron storage [Figure 7.1, Weiss et al. 1997]. Additionally, stimulation with IFN-γ/LPS also causes downregulation of TfR mRNA which causes decrease in uptake of iron [Figure 7.1, Weiss et al. 1997]. On the contrary, stimulation by IL-4 and IL-13, factors responsible for M2 polarization, caused reversal of IFN-γ/LPS stimulation on in macrophages i.e. upregulation of ferritin synthesis and TfR mRNA, culminating in increased iron uptake and storage [Figure 7.1, Weiss et al. 1997]. Consequently, these iron loaded macrophages then lose their ability to kill intracellular pathogens like *Mycobacteria*, *Salmonella* and *Leishmania* [Reviewed in Recalcati et al. 2012, Das et al. 2009].

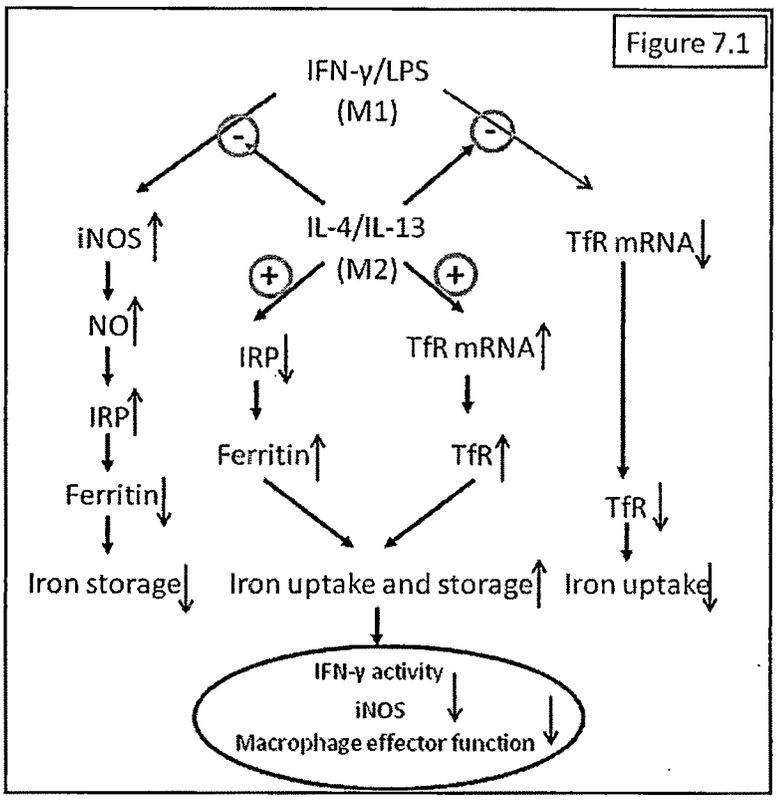

Figure 7.1
Regulation of iron metabolism in macrophages stimulated with either IFN-γ/LPS or IL-4/IL-13. Increased NO mediated by IFN-γ/LPS stimulation caused downregulation of ferritin translation by subsequent IRP activation. It also downregulates TfR mediated iron uptake. IL-4/IL-13 reversed the phenomena as they suppress IRP activation resulting in increased ferritin synthesis. Alongside IL-4/IL-13 induces activation of TfR transcription, ultimately downregulates macrophage effector function with increased iron uptake and storage.

Study objectives

In human leishmaniasis studies have revealed decreased circulating levels of iron in both cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) and this deficiency correlated with the nutritional status of the host [Kocyigit et al. 1998, Lal et al. 2012]. However studies regarding role of iron in PKDL are till date not available and as we have observed that circulating monocytes and dermal macrophages in PKDL are alternatively activated i.e M2 macrophages, we felt it pertinent to study the regulation of iron by its related genes PKDL. We have measured the plasma levels of iron, transferrin, ferritin along with intramonocyte accumulation of iron, surface expression of CD71 (transferrin receptor), along with the mRNA expression of ferritin, transferrin, transferrin receptor, CD163 (haptoglobin-haemoglobin receptor), HO-1 (Heme oxygenase 1) from monocyte enriched PBMCs and from lesional sites.

Immunophenotyping of peripheral blood leukocytes

Peripheral blood (100 µl) was surface stained with fluorochrome conjugated antibodies to CD14 Fluorescein isothiocyanate or FITC, CD71 Allophycocyanin or APC, along with appropriate isotype controls as described in chapter 2.

Isolation of peripheral blood mononuclear cells (PBMC) and monocytes

Peripheral blood was carefully layered over HiSep 1073 (1:1) for isolation of monocytes and centrifuged (400 g x 30 min). The PBMC-rich or monocyte rich interface was washed twice in phosphate buffered saline (PBS, 0.01 M, pH 7.2) and resuspended in RNALater solution for studying mRNA expression or used for intramonocyte iron pool measurement.

Isolation of RNA and Reverse transcriptase-PCR from peripheral blood mononuclear cells and lesional sites

Total RNA was isolated from monocyte enriched PBMCs ($1x10^6$ cells) or from lesions according to the manufacturer's instructions Reverse transcriptase-PCR was performed on isolated RNA (50 ng) with a one-step reverse transcriptase-PCR kit using gene-specific primers for ferritin, transferrin, transferrin receptor, HO-1, CD163 and β-actin as described in chapter 2.

Determination of intracellular iron in monocytes

Monocytes ($5x10^5$/ml) were centrifuged (400 g X 5 minutes), resuspended in 500 µl PBS, stained with Calcein-AM (1.25 nM) for 30 minutes at 37°C and fluorescence was acquired on a flow cytometer.

Measurement of plasma Iron, ferritin and Transferrin

Plasma level of iron was measured according to manufacturer's instructions. Briefly, plasma (100 µl) was treated with an acidic buffer. Then, ferrozine was added to the sample to form a violet coloured complex whose absorbance was measured at 570 nm. The concentration of iron in plasma was calculated against a supplied standard (100 µg/dl). Plasma ferritin level was measured by an enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions while Plasma Transferrin was measured by an immunoturbidometric method.

Results

Accumulation of intracellular iron in monocytes from PKDL

To investigate the levels of iron in monocytes from patients with PKDL we measured the intracellular labile iron pool by flow cytometry using a fluorescent probe Calcein-AM, whose fluorescence is known to be quenched by Fe^{+3}. Accordingly, the fluorescence of Calcein is inversely proportional to the intracellular Fe^{+3}. Under basal conditions, the labile iron pool was much higher in patients with PKDL than healthy controls, the fluorescence of Calcein being 85.49 ± 13.18 vs. 186.8 ± 27.43, $p<0.05$ (Figures 7.2A and B); treatment caused no change in fluorescence (74.64 ± 12.64, Figures 7.2A and B). Subsequently, to analyze whether this accumulated iron within monocytes was a fraction of the systemic iron pool i.e. plasma iron, we measured levels of plasma iron in patients with PKDL and showed it was significantly lowered when compared to healthy controls (54.99 ± 27.90 µg/dl vs. 322.2 ± 94.40 µg/dl, $p<0.05$, Figure 7.2C). Treatment caused a moderate but nonsignificant increase in plasma iron (110.50 ± 56.13 µg/dl, Figure 7.2C).

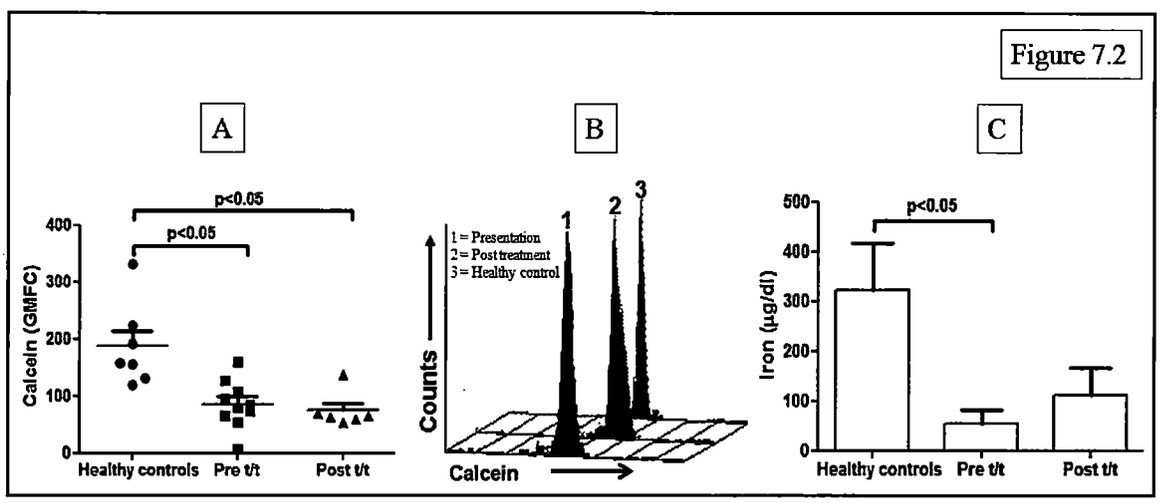

Figure 7.2
A. Scatter plot for intracellular iron pool in monocytes from healthy controls (n = 7, •), patients with PKDL at presentation (Pre t/t, n = 10, ■) and post treatment (Post t/t, n = 7, ▲).
B. A representative flow cytometric diagram of GMFC of calcein in monocytes from a healthy control (3) patient with PKDL at presentation (1) and at the end of treatment (2).
C. Plasma iron level in healthy controls, patients with PKDL at presentation (Pre t/t) and after treatment (Post t/t).

PKDL was associated with altered levels of ferritin, transferrin and transferrin receptor

Ferritin is one of many iron-containing proteins whose expression is down regulated in M1 macrophages through the effect of NO on iron response factors [Stafford et al. 2002]. Extracellular ferritin has been reported to act as a pro-inflammatory signaling molecule in hepatic stellate cells where it induces inducible nitric oxide synthase (iNOS), IL-1β and NF-κB suggesting that apart from its role in iron binding, it also plays an important role in immunity [Ruddell et al. 2009].

In PKDL patients we have demonstrated that levels NO are decreased in monocytes (Figure 4.4A, Chapter 4) which would influence the ferritin status. At the lesional site, an enhanced mRNA expression of ferritin was evident and was non-detectable in healthy controls (1.60 ± 0.49, Figure 7.3A); with treatment, its expression was significantly increased by 3.4 fold (5.41 ± 0.33, $p<0.001$, Figure 7.3A). Akin to lesional expression, in circulating monocytes the mRNA expression of ferritin was significantly increased at presentation as compared to healthy controls where it was undetectable (7.38 ± 2.14, $p<0.01$, Figure 7.3B). Treatment caused a 2.9 fold, yet non-significant decrease in the expression of ferritin (2.54 ± 1.22, Figure 7.3B). The plasma levels of ferritin were significantly lowered than controls (45.45 ± 9.39 mg/dl vs. 98.22 ± 16.61 mg/dl, $p<0.05$, Figure 7.3C) which marginally increased with treatment (55.01 ± 11.17 mg/dl, Figure 7.3C).

Transferrin is a serum protein primarily involved in the transport of iron (in Fe^{+++}) throughout the body. Additionally, tansferrin is produced within activated macrophages and binds to intracellular iron which may limit the availability of intracellular iron (Fe^{+++}) for intracellular pathogens [Stafford et al. 2002]. With regard to mRNA expression of lesional transferrin, a significant 7.5 fold increase at presentation was observed (7.52 ± 0.85, Figure 7.3A) which decreased significantly following treatment (2.31 ± 0.59, $p<0.01$, Figure 7.3A). When we analyzed the mRNA expression of transferrin in circulating monocytes, no changes were present either at disease presentation (2.64 ± 1.33) or at post treatment (3.60 ± 1.57) when compared with healthy controls (2.26 ± 1.15, Figure 7.3B). With regard to plasma transferrin, we found a significant rise in transferrin level at disease presentation as compared to healthy controls (240.60 ± 13.56 mg/dl vs. 182.50 ± 11.53 mg/dl, $p<0.05$, Figure 7.3C) which reverted to levels compatible with controls following treatment (197.30 ± 16.86 mg/dl, Figure 7.3C).

Transferrin is taken up via the transferrin receptor (TfR, CD71) whose expression get downregulated following IFN-γ/LPS stimulation and conversely its expression is upregulated by Th2 derived cytokines [Walsh et al. 2005]. In PKDL where the cytokine response is biased towards a Th2 type (Chapter 4, Figure 4.2), the mRNA expression of TfR in lesions was

significantly increased by 13.5 fold while it was undetectable in healthy controls (13.59 ± 1.32, $p<0.001$, Figure 7.3A); curtailment of TfR was evident after treatment (3.88 ± 1.26, $p<0.05$, Figure 7.3A). On the contrary, the expression was pattern in circulating monocytes was opposite from dermal site as a 2.6 fold decrease in the mRNA expression was present at disease presentation when compared with healthy controls (3.08 ± 0.36 vs. 7.80 ± 3.26, Figure 7.3B), treatment caused the expression increased to 5.92 ± 1.60 (Figure 7.3B). Flow cytometric evaluation of the surface expression of TfR at presentation showed, its expression was 3 fold higher at presentation *vis a vis* than healthy controls (Mean ± SEM being 19.20 ± 5.24 vs. 6.32 ± 1.62, Figure 7.3D). Following treatment, the expression of CD71 was decreased 2 fold (9.01 ± 3.05, Figure 7.3D).

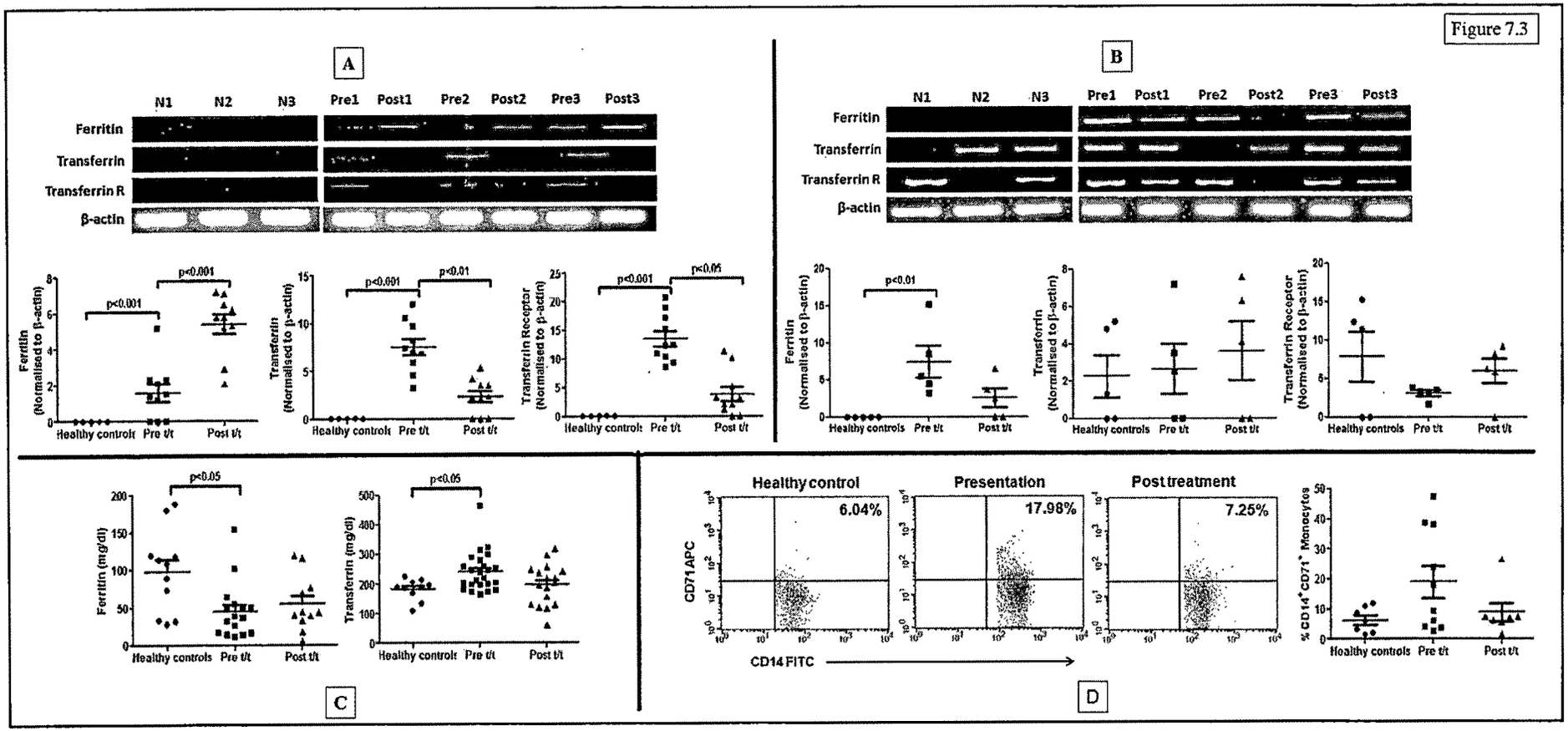

Figure 7.3

A. Representative profile of mRNA expression of ferritin, transferrin, transferrin receptor and β-actin in lesions isolated from patients with PKDL before (Pre1-Pre3, n = 3) and after treatment (Post1-Post3, n = 3) and from healthy individuals (N1-N3, n = 3) and scatter diagram for expression of ferritin, transferrin, transferrin receptor after normalization to β-actin levels in healthy controls (n =5), patients with PKDL at presentation (Pre t/t, n =10) and after treatment (Post t/t, n =10) was quantified by densitometric analysis of RT-PCR products.

B. Representative profile of mRNA expression of ferritin, transferrin, transferrin receptor and β-actin in monocyte enriched isolated from patients with PKDL before (Pre1-Pre3, n = 3) and after treatment (Post1-Post3, n = 3) and from healthy individuals (N1-N3, n = 3) and scatter diagram for expression of ferritin, transferrin, transferrin receptor after normalization to β-actin levels in healthy controls (n =5), patients with PKDL at presentation (Pre t/t, n =5) and after treatment (Post t/t, n =5) was quantified by densitometric analysis of RT-PCR products.

C. Plasma ferritin, transferrin levels in healthy controls, patient with PKDL at presentation (Pre t/t) and after treatment (Post t/t).

D. Representative profiles of surface expression of CD71 (transferrin receptor) on monocytes from healthy controls, patient with PKDL at presentation and after treatment. Scatter diagram of CD71 expression in healthy controls, patients with PKDL at presentation (Pre t/t) and after treatment (Post t/t).

Expression of CD163 and HO-1 in PKDL

CD163, the scavenger receptor for haptoglobin-haemoglobin complex is considered as one of the most potent markers of alternatively activated/M2 macrophages [Reviewed in Vega and Corbi 2006]. CD163 is known to induce Heme oxygenase-1 and thus suppresses inflammation. Alongside CD163 is considered responsible for the increased labile iron pool (LIP) of M2 macrophages. At the lesional site, the expression of CD163 was significantly raised in patients with PKDL than healthy controls where expression was undetectable (8.02 ± 0.83, Figure 7.4A); following treatment the mRNA expression was decreased by 10.4 fold (0.77 ± 0.36, $p<0.001$, Figure 7.4A). In circulation, the scenario was similar as CD163 was elevated in PKDL patients as compared to healthy controls (10.37 ± 3.21, vs. 1.82 ± 0.56, Figure 7.4B) and curtailment was evident after treatment (6.84 ± 2.28, Figure 7.4B).

Heme oxygenase-1 is inducible in macrophages in response to iron loading as well as stress and cytokines like IL-10 [Cairo et al. 2011]. It degrades heme to carbon monoxide (CO), biliverdin and iron. Biliverdins after conversion into bilirubin along with CO have a potent anti-inflammatory action. CO suppresses LPS induced activation of IL-1, IL-6, TNF-α and NO in monocytes and macrophages by preventing degradation of IκBα [Vega and Corbi 2006]. Additionally CO can induce IL-10 production in macrophages. In PKDL, we found a significant 15.87 fold increase in mRNA expression of HO-1 in disease presentation at the lesional sites (15.87 ± 1.32, Figure 7.4A) which significantly decreased following treatment (0.96 ± 0.42, $p<0.001$, Figure 7.4A). Similarly, in circulating monocytes, the mRNA expression of HO-1 was 5 fold higher in PKDL patients than healthy controls (46.68 ± 9.65 vs. 9.43 ± 1.99, $p<0.05$, Figure 7.4B) but regressed only marginally with treatment (33.43 ± 10.90, Figure 7.4B).

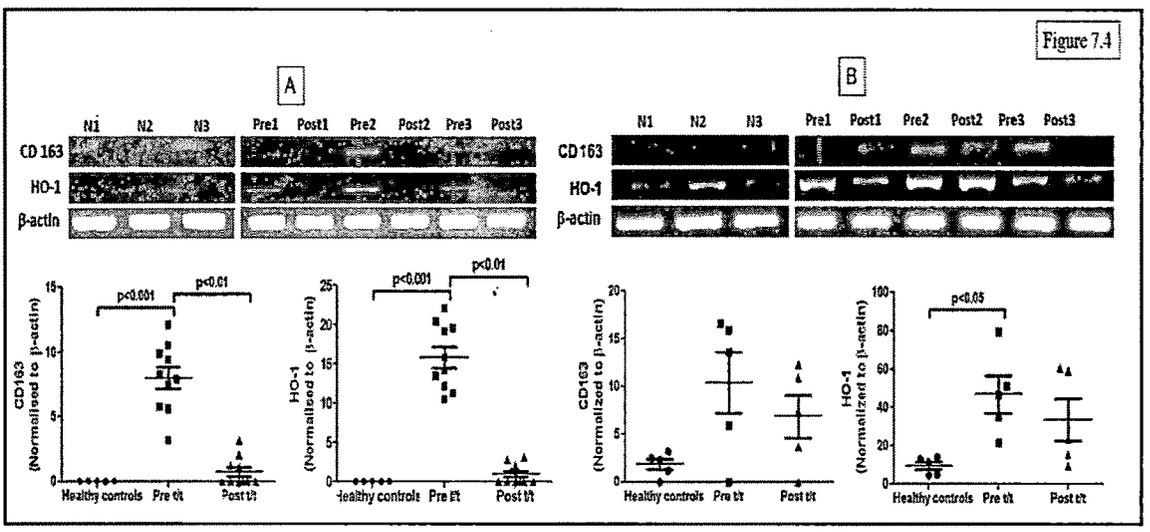

Figure 7.4:
A. Representative profile of mRNA expression of CD163, HO-1 and β-actin in lesions isolated from patients with PKDL before (Pre1-Pre3, n = 3) and after treatment (Post1-Post3, n = 3) and from healthy individuals (N1-N3, n = 3) and scatter diagram for expression of CD163, HO-1 after normalization to β-actin levels in healthy controls (n =5), patients with PKDL at presentation (Pre t/t, n =10) and after treatment (Post t/t, n =10) was quantified by densitometric analysis of RT-PCR products.
B. Representative profile of mRNA expression of CD163, HO-1 and β-actin in monocyte enriched PBMCs isolated from patients with PKDL before (Pre1-Pre3, n = 3) and after treatment (Post1-Post3, n = 3) and from healthy individuals (N1-N3, n = 3) and scatter diagram for expression of CD163, HO-1 after normalization to β-actin levels in healthy controls (n =5), patients with PKDL at presentation (Pre t/t, n = 5) and after treatment (Post t/t, n = 5) was quantified by densitometric analysis of RT-PCR products.

Discussion

Maintenance of cellular iron homeostasis is not only a general prerequisite for the growth and proliferation of all cells but is also of central importance for the regulation of immune function. Besides its role in lymphocyte proliferation, iron availability strongly influences cell mediated immune effector mechanisms involving macrophages [Weiss et al. 1995]. In mammals, cellular uptake, storage and consumption of iron are largely coordinated by post-transcriptional regulation. Two cytoplasmic proteins iron regulatory protein-1 (IRP-1) and IRP-2, interact with specific mRNA stem loop structures called iron responsive elements (IREs). During iron deficiency or oxidative stress or increased generation of NO, IRP binding to IREs located within the 5' untranslated region (UTR) of ferritin mRNA causes translational repression and decreased ferritin expression. Additionally, the high affinity interaction between IRP and IREs within the 3' untranslated region of the mRNA for the TfR, the major receptor for cellular iron, increases the stability of this mRNA by protecting it from mRNA degradation, and promotes iron uptake into cells [Weiss, 2002].

Under inflammatory conditions, both Th1 and Th2 derived cytokines, short lived radicals and acute phase proteins affect iron metabolism both by IRP/IRE dependent and independent pathways leading to changes in iron homeostasis. IFN-γ and LPS downregulate

translation of ferritin via induction of NO and subsequent IRP activation along with downregulation of TfR expression via an IRP independent pathway the net result being lowered LIP. On the contrary IL-4 and IL-13 enhances ferritin expression in M2 macrophages by inhibiting formation of NO, following deactivation of IRP resulting in increased LIP. At the same time, TfR mRNA expression is increased, most likely due to unblocking of the inhibitory IFN-γ/LPS signal on TfR expression (Figure 7.1). Thus IL-4 and IL-13 increases iron uptake and storage by macrophage which negatively affect the effector functions of macrophages and facilitates development of an anti-inflammatory milieu [Reviewed in Theurl et al. 2005, Stafford et al. 2002, Weiss et al. 1997].

In PKDL, we have demonstrated that plasma IL-4, IL-10, TGF-β and IL-13 are raised and that resulted in polarization of circulating monocytes and dermal macrophages towards M2 type (discussed in chapter 4 and 6). Here, we have assessed the iron homeostasis in circulating monocytes and lesions from patients with PKDL. In PKDL, we have found an increased intramonocyte labile iron pool using the Calcein-AM assay which is sustained even after treatment (Figures 7.2A and B). It is reported that the LIP is larger in M2 macrophages and in PKDL, macrophages are predominantly of M2 type. Increased storage of iron in turn affects generation of NO and other effector functions of macrophages. Similarly in PKDL we observed that at presentation generation of NO is impaired along with downregulation of co-stimulatory molecules and synthesis of pro-inflammatory cytokines (Chapter 5, Figures 5.3 and 5.5). Although reports regarding intra-macrophage iron in M1 and M2 cells are conflicting, [Reviewed in Cairo et al. 2011, Recalcati et al. 2012, Corna et al. 2010] it may be due to the multiple sources of the macrophages. Mouse macrophages cannot mimic the human system as also differentiation of human monocyte derived macrophages by different cytokine(s) do not necessarily match the *in vivo* scenario [Reviewed in Babu et al. 2009, Boelaert et al. 2007]. In tuberculosis, it has been observed that alternatively activated macrophages expressing arginase decreased NO production resulting in deceased efflux of iron [Reviewed in Boelart et al. 2007]. As PKDL is also a chronic infectious disease we observed similar changes. Additionally, plasma levels of iron are decreased probably owing to increased uptake by alternatively activated monocytes [Figure 7.2C].

Our next objective was to identify the possible pathway(s) by which plasma iron was taken up by the phagocytic cells, and accordingly we have measured plasma ferritin and transferrin along with mRNA expression for ferritin, transferrin and TfR in circulating monocytes and in lesional biopsies. Plasma ferritin level was found to be decreased which further proved that the plasma iron was not stored in ferritin [Figure 7.3C]. On the contrary, plasma transferrin was significantly increased; implicating that iron is most likely transported via transferrin-TfR pathways [Figure 7.3C]. As in PKDL the circulatory cytokine milieu was biased towards Th2 type (Chapter 4), we assessed the mRNA expression for ferritin and

transferrin receptor along with transferrin. In PKDL, the mRNA expression of ferritin and TfR in lesional biopsies were significantly increased at presentation and decreased following treatment (Figure 7.3 A), whereas in circulation, mRNA expression of ferritin was increased but not TfR (Figure 7.3B). The surface expression of TfR (CD71) was increased at presentation although not significantly and decreased with treatment (Figure 7.3D). Our observation led us to conclude that circulating monocytes and dermal macrophages are alternatively activated at disease presentation which resulted in downregulation of IRP along with subsequent upregulation of ferritin and TfR. This led to an increased iron uptake and storage that contributed to the decreased macrophage effector function.

Besides iron itself, enzymes and metabolic products of iron metabolism in macrophages are also capable of modulating the release of pro-inflammatory and anti-inflammatory cytokines, thus fine tuning the state of macrophage activation [Vega and Corbi 2006]. Uptake of haptoglobin-haemoglobin via CD163 induces Heme oxygenase 1 in monocytes/macrophages which degrades heme into iron, CO and biliverdin. Both CD163 and HO-1 are characteristic of the M2 metabolic signature and occurs not only in response to increased availability of heme but also in response to M2 polarizing agents including IL-10 [Lee and Chau 2002]. HO-1 counteracts inflammation and thus can contribute to the biological properties of M2 macrophages [Cairo et al. 2011]. HO-1 catalyzed generation of CO is known to suppress TLR-4 signaling and also blocks the LPS (Lipopolysaccharide) mediated induction of pro-inflammatory cytokines by modulation of mitogen activated protein kinase (MAPK) pathway. In PKDL, as we have found decreased expression of TLR-4 (chapter 5, Figure 5.2) along with decreased phosphorylation of MAPKs upon LPS induction (unpublished data) it supports this hypothesis. Here we have observed significant upregulation of both CD163 and HO-1 at lesional site as well as in circulating monocytes (Figures 7.4A and B) which regressed with treatment. HO-1 catalyzed generation of CO also decreased superoxide generation and increased reduced glutathione. Likewise in PKDL we have also observed within circulating monocytes , a decrease generation of superoxide along with an increased levels of intracellular glutathione (Chapter 5, Figures 5.4D,E,F and G) thus highlighting the role of CD163 mediated iron uptake and subsequent upregulation of HO-1 in PKDL.

Take together, in this study we have established the role of iron metabolism in macrophages from patients with PKDL. Our study revealed that increased iron loading associated with M2 macrophages in PKDL plays an important role in supporting disease persistence and parasite survival and therefore could be considered as a potential immunomodulatory target in leishmaniasis.