Chapter 6

Discussion
Regulation of virulence mechanism/s in *Leishmania*

According to the model proposed for the *Leishmania* virulence it is possible to regulate the virulence by differential expression of qualitative/quantitative differences of the invasive/evasive and pathoantigenic determinants. Here I am considering virulence as a clinical outcome of infection in the form of different pathology of leishmaniasis. Therefore differential regulation of either of these determinants may alter the outcome of infection. *Leishmania* virulence factors are mainly responsible for virulent phenotype of *Leishmania*. These factors can be divide into three major categories if they can regulate the virulence:

1. Positive factors of virulence
2. Negative factors which reduces virulence
3. Facultative factors which normally do not participate in virulence but do so when a positive contributor is absent.

A complex interaction of these three groups of factors may give rise to different outcome of virulence. Different interactions between these groups and their effect on virulence have been shown in Fig 6.1. Normally virulence phenotype represents the balanced interactions of positive and negative factors with no influence of facultative factors (Fig 6.1 1st & Vth row). Any loss of positive factors either by deletion or silencing may decrease the virulence (Fig 6.1 IInd row). Loss of positive factor not always lowers the virulence. If the function of this lost factor can be compensated by another positive factor shown by "++" sign or by any facultative factors (Fig 6.1 IIIrd & IVth row) virulence can be regain to its normal phenotype. Another scenario is the loss or down regulation of a negative factor. This will accompanied by an increase in virulence or hyper virulence (Fig 6.1 VIth row). There is only one example of negative factor in *Leishmania* till date where loss of pteridine reductase 1 (PTR1) gene increases the virulence of *L. major* (Cunningham et al., 2001).

Tampering with these determinants will give us the tools to deal with leishmaniasis. Developing specific inhibitors of the invasive/evasive determinants to prevent infection or producing infective but non-pathogenic mutants deleting or
altering the genes that encode for pathoantigenic determinants may help in that direction.

![Diagram of virulence factors](image)

**Figure 6.1 Regulation of virulence in Leishmania.** Blue, green and yellow squares with “+” are positive factors of virulence; pink square with “−” are negative factors and blank red boxes are facultative factors.

**Role of DRGs in attenuation**

DRG1 over expression causes increased survival of virulent promastigote that is similar to the attenuated line of parasites whereas DRG2 over expression causes early death which is very similar to growth defect of VPS4 mutant of *Leishmania* with incomplete metacyclogenesis i.e. low virulence. Here it can be speculated that DRGs have some role in attenuation of the parasite. The probable role of DRGs may have some cryptic mechanism. Here I hypothesize that it may be due to a defect in endosomal/lysosomal pathway or altered autophagy.

If over expression of these DRGs can cause the lowering of the virulence then knockout of these genes may put the *Leishmania* cells in state where it will show hypervirulence. A variety of anti-virulence genes have been identified in pathogens whose loss confers the increased virulence (Foreman-Wybert and Jeff F. Miller, 2003; ten Bokam et al., 2008). Genes belong to this category whose disruption causes hypervirulence in pathogens are referred as antivirulence gene. It is thought that anti-virulence genes offer some benefit to pathogens as they have maintained
these loci throughout evolution. The conservation of DRGs sequences within the trypanosomatids indicates that during evolution these DRG sequences are kept intact without any mutations. For some pathogens, less virulent organisms might favor host survival and thus transmission to susceptible hosts. Hypervirulent mutants of *Leishmania major* are also observed where knockout mutation in the *ptr1* gene results in increased virulence in mice (Cunningham et al., 2001). *ptr1* encodes the enzyme pteridine reductase 1, which allows *Leishmania* to salvage reduced pteridines. Hypervirulence in BALB/c mice manifested as increased lesion formation, and lesions contained increased parasite numbers. It was hypothesized that PTR1 functions to increase the survival of the infected mammalian host, thus increasing transmission to the sand fly vector and facilitating spread of *L. major*.

In tissue culture system, *Leishmania* has gone through several cycles of log and stationary phase that resembles to metacyclogenesis by so many means but still *Leishmania* parasite loses virulence. It is well argued that the genetic factors which control metacyclogenesis and virulence are not necessarily stably expressed. In addition, some anti-virulence genes might have been retained because they promote survival in a non-host environment. This non-host environment is similar to our tissue culture conditions where *Leishmania* cells never interacts with the host cells and during long term culture they have to induce the expression of their anti-virulence genes. But they may lose their virulence in the cost of their survival. It is also proposed that the evolutionary success of a pathogen requires the efficient iteration of its life cycle (Brown et al., 2006). Similarly loss of lpg2 in *L. major* causes the no pathology but increased persistence/survival in the mice (Spath et al., 2003). DRG1 also increases the survival of the *Leishmania* parasite at least in the in-vitro culture conditions. Whether it is also behaves similarly in-vivo that needs to be examined. These defects in virulence but not in the persistence have provided a tool to the study of persistent *Leishmania* infection and to develop anti-parasitic vaccines. Investigators had tried numerous approaches to develop safe non-live vaccine against *Leishmania* using several recombinant *Leishmania* antigens including GP63, LACK, PSA-2, PFR2, A2 and HASPB1. Drug treated attenuated parasites; recombinant *Leishmania* expressing cytokines and DNA based vaccines...
were also tried. But these strategies did not show complete long-lived protection. Attenuated forms of other pathogen such as intracellular micro-organisms and helminthes are shown to be highly effective vaccine (Hess et al., 2000). Therefore vaccine composed of avirulent or attenuated parasites holds the promise for an anti-*Leishmania* vaccine (Handmann, 2003).

The *Leishmania PTR1* and lpg2 gene are involved in host survival either by limiting the virulence or by increased persistence of the *Leishmania* into the host cell therefore transmission of parasite. They have presented the evidence of different class of genes in *Leishmania* that limit their virulence. DRGs might be an addition to this list. The mechanisms how these genes down regulate the virulence and increase the survival of the parasite into the host are still an open question. More knowledge of this class of genes may help in understanding their role in reactivation of visceral leishmaniasis in immune-compromised patients.