THE ROLE OF THE LEPTIN RECEPTOR ON T CELLS IN *HELIcobacter pylori* INFECTION AND CLEARANCE IN MICE

by

DOUGLAS S. EMANCIPATOR

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Department of Biology

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CASE WESTERN RESERVE UNIVERSITY

SCHOOL OF GRADUATE STUDIES

We hereby approve the thesis/dissertation of

__Douglas S. Emancipator______________________________

candidate for the ___Master's (MS)_______________degree *.

(signed)____James Zull____________________________________

(chaired of the committee)

___Nancy DiIulio, Ph.D.__________________________

___John Nedrud, Ph.D.____________________________

___Radhika Atit, Ph.D.___________________________

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(dates) __6/25/2008____________

*We also certify that written approval has been obtained for any
proprietary material contained therein.
Dedication

This work is dedicated to:

- Tina, my loving wife, who has supported me through the entire process. Thank you for believing in me, I love you.

- My parents, Steven and Judy Emancipator, thank you for everything, but especially helping me see I can accomplish any goal I work for and instilling in me the drive to do so.
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Helicobacter pylori (H. pylori) infects the stomach of over 50% of the World’s population and often results in gastritis and ulcers. In addition, the World Health Organization has categorized H. pylori as a class I carcinogen. Still, little is known about how H. pylori induced gastritis arises and resolves. The adaptive immune response to H. pylori shapes proclivity to gastritis and clearance of infection. Recently, interaction of Leptin with its receptor has been recognized to modulate immune responses, and particularly reduces the T cells ability to incite inflammation. We hypothesized that the LepR modulates the adaptive immune response and thereby plays a significant role in the clearance of H. pylori from gastric mucosa. The data indicate that the LepR on CD4+ T cells is required for clearance of H. pylori from infected SCID mice adoptively transferred with T cells.
1. Introduction
Since the initial description associating *Helicobacter pylori* (*H. pylori*) infection of the gastric mucosa with gastritis and peptic ulcers in 1983 (16), a great body of knowledge has accumulated about *H. pylori*. However, *H. pylori* infection still has a prevalence of over 50% worldwide and in many populations, particularly in developing nations, the prevalence is over 90% (10). In addition, a strong association with gastric cancer has been recognized, and the World Health Organization has categorized *H. pylori* as a class I human carcinogen (5, 35). Although much is known about the genetics and biology of *H. pylori*, and some virulence mechanisms have been identified, we still know little about how *H. pylori* induced gastritis arises and resolves. Moreover, there is only skeletal information on the regulation of immune responses to *H. pylori*. Infection by *H. pylori* ordinarily leads to a Th1 mediated response that is CD4+ T cell dependent (1, 6). The Th1 response is cell mediated and associated with a high amount of Interferon gamma (IFN-γ), which results in inflammation. Although *H. pylori* infection is accompanied by local inflammation and evolution of *H. pylori*-specific adaptive immune responses, these mechanisms typically fail to eradicate *H. pylori* (2, 3, 12). Inflammation due to *H. pylori* infection is dependent upon CD4+ T cells (8, 29). Under certain circumstances, prophylactic intra nasal immunization of mice with *H. pylori* lysate and adjuvant can protect against colonization (12). However, the adjuvants employed in these studies cannot be used in humans due to their adverse effects. Currently no commercial vaccine or immunization is available for humans.

Our interest in leptin stems from previous studies that demonstrate an adipocyte or “fat cell” signature associated with a protective vaccination against *H. pylori* (23, 33). Leptin is a protein most well know as a adipocyte hormone molecule critical in regulating
energy, hunger, and body weight. Leptin also has been shown to affect the immune system by affecting T cells (14, 15, 17-19, 21, 22, 24, 30). The leptin receptor (LepR, ObR) is one among a group of class I cytokine receptors. Siegmund et. al. showed that mice unable to produce leptin or its receptor are resistant to experimental models of colitis (31). In this model, the diminished ability to produce an inflammatory response correlates to a decrease in cytokine production and an increased level of apoptosis of lamina propria lymphocytes. Further work has shown that this is a result of the Leptin receptor on T lymphocytes which reduces the T cells ability to incite inflammation (31).

Given the observed effects of leptin on T cell functions, we recognized a novel opportunity to study the regulation of immune responses to *H. pylori* infection of the gastric mucosa. We hypothesized that leptin, acting through its receptor (LepR), modulates the adaptive immune response, particularly in the CD4+ compartment, and thereby plays a significant role in the clearance of *H. pylori* from gastric mucosa. The colitis model utilized by Siegmund and colleagues to demonstrate a role for the leptin receptor in colitis is based on a model originally developed by Powrie (27, 28). Eaton and colleagues modified this model to show that adoptive transfer of T cells into H. pylori infected SCID mice results in clearance of H. pylori (8, 9). We combined these two models by adoptively transferring CD4+ T cells from “Lean” (LepR +/+ or +/-) or Leptin receptor -/- mice into H. pylori infected SCID recipients (26-28). SCID mice have normal levels of leptin and leptin receptors on non lymphoid cells. In addition, we sought to measure the influence of the LepR on selected critical T cell functions (proliferation and cytokine synthesis) by measuring differences in these parameters between SCID recipients of LepR +/- compared to Lean cells when exposed *in vitro* to *H. pylori* lysate.
Finally, we planned to ascertain any differences in quantities of T cell-derived cytokines in cells isolated from stomach tissue.

2. Materials and Methods

2.1 Animal Preparation

The three different strains of female mice, SCID (C.B-17/ICr Hsd-Prkdc-scid, Harlan), Obese, LepR/- (Hsd: BKS. Cg-+/+Leprdb/Leprdb/OlaHsd) which is an autosomal recessive mutation on chromosome 4 (Harlan) and the “lean” littermates (Hsd: BKS. Cg-m+/+Lepr+db/olaHsd), which were a combination of homozygous wild type and heterozygotes, were used as controls. All mice were purchased from Harlan (Harlan, Indianapolis, Indiana) and arrived at 3-5 weeks of age, free of pathogens including Helicobacter spp. Upon arrival, the mice were allowed to acclimatize to the environment for at least one week before starting the experiment. The mice were housed in autoclaved microisolator cages with autoclaved water and standard Teclad chow (Harlan Teklad, Indianapolis, Indiana) ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Case Western Reserve University.

2.2 Bacteria and Bacterial Products

*H. pylori*, Sydney strain (SS1) (11, 13), obtained from a long standing culture in the lab were grown on Columbia agar plates containing 7% defibrillated horse blood and antibiotics (20ug/ml Trimethoprim, 6ug/ml Vancomycin, 16ug/ml Cefsolodin, 2.5ug/ml Amphotericin). The cultures were incubated for one week in anaerobic jars (Becton Dickinson) at 37°C with a CampyPakPLUS pack (Becton Dickinson) to create a microaerobic, low O₂ atmosphere.
To challenge the mice, *H. pylori* were passed to a liquid culture containing Brucella broth (Difco), 10% fetal bovine serum (Gibco) and antibiotics. The flasks were incubated at 37°C in an incubator with 10% CO2 for 24 hours. Bacteria were checked for viability by visual observation (quantity, size and motility) and concentration by checking light absorbance at 450nm (0.09 to 0.1 above media)(12). *H. pylori* were administered by oral gavage giving each animal 0.5 ml of Brucella broth containing a concentration of 10^8 colony forming units (cfu)/ ml of *H. pylori*. At the time of challenge, an aliquot of the liquid culture was also cultured back onto complete blood agar plates to determine quantitative cfu/ml. Challenge was repeated within 2 days to ensure colonization of bacteria.

The *H. pylori* antigen used during in vitro studies was prepared from plate cultures. *H. pylori* harvested by gently scraping with a “Lazy-L spreader” (Sigma) from the plates with PBS. The bacteria was then centrifuged for 20 min at 5000 rpm and then resuspended in 2ml of Dulbecco’s PBS and lysed by 4 x 30 second blasts using a probe sonicator (Sonics & Materials Inc., Danbury, CT). The sonicate was then spun at 5000 rpm for 20 min to remove any unlysed bacteria and large particles. The supernatant was then passed through a 0.45μm filter and the final protein concentration was calculated by Bradford assay (Pierce).

2.3 Experimental Design

SCID mice were randomly assorted into one of 4 groups, the first group (unchallenged) served as non infected controls challenged with sterile medium, the remaining three groups of SCID mice were challenged with the SS1 strain of *Helicobacter pylori*. After four weeks of infection animals in groups 2-4 were injected
with sterile PBS or underwent adoptive transfer of CD4+ T cells isolated from the spleens of LepR-/- mice or control Lean littermates. Four weeks post adoptive transfer mice were sacrificed and tissue collected for study. Blood was withdrawn and plasma isolated and frozen at -80°C. Spleens and three section of stomach containing a region from the cardiac through the pylorus along the greater curvature were taken and weighed. The first section of stomach was used to quantify colony forming units (cfu), the second was used for histological review of gastritis, and the third frozen at -80°C for PCR evaluation of cytokines.

2.4 Quantification of Bacteria in Mouse Stomach
Bacteria were quantitatively cultured from mouse stomach sections. A preweighed section of stomach tissue was homogenized in 200μl of Brucella broth containing 10% fetal bovine serum with a 1.5ml disposable tissue grinder and pestel (Kontes, Vineland, NJ). A serial dilution was made of the homogenate and cultured in the same way as above with the addition of 20μg/ml of Bacitracin to aid in suppression of other natural flora. Calculations were made from colony counts (log(200μl x count x dilution factor x 100)/g tissue)) to determine log cfu’s per gram tissue (wet weight). Colonies were also tested for urease, oxidase, and catalase activity to confirm *H. pylori* identification (12).

2.5 Splenocyte and CD4+ T cell Isolation
Spleens from LepR -/- and lean control mice were collected and placed in ice cold, sterile PBS. Cell suspensions were made using the plunger of a 5ml disposable syringe to break up the tissue. The cell suspension was then filtered through a 70μm cell
strainer and the erythrocytes lysed with ammonium chloride lysis solution (8.3g/L ammonium chloride: 20.59g/L Tris, pH 7.65 in a 9:1 ratio respectively). Splenocytes were washed and resuspended in medium. Cells were then counted with a hemocytometer to determine concentration and Acridine Orange was used to determine viability. Splenocytes used for adoptive transfer were fractionated to isolate CD+4 T cells. Cells were incubated with anti-CD4 microbeads (CD4 (L3T4) microbeads, Miltenyi Biotec, Auburn, CA) and passed through a MACs column. The column was then washed with buffer once, then removed from the magnetic column and media was forced through releasing the captured CD4+ cells. Cells were then counted to check viability and to adjust the final concentration of 1x10^6 cells/200μl and then transferred into select groups of SCID mice by intraperitoneal injection and control mice were injected with sterile saline solution (4, 9).

2.6 Cell Proliferation
Unfractionated splenocytes were isolated as described above from spleens collected at harvest. Cells were cultured in triplicate at a final concentration of 1x10^5 cells/well for 48 hours in 96 well plates with complete 1640 RPMI medium (Gibco) with 1%Penecilin-Streptomycin and 10%FBS and stimulated with *H. pylori* lysate (12.5μg/ml), Phytohemagglutinin (PHA) (10μg/ml) or no stimulus. After incubation cells were spun down and supernatants were collected for cytokine analysis. The cells were then resuspended in fresh complete 1640 RPMI (Gibco) medium without phenol red and 20ul of CellTiter 96 Aqueous One solution (Promega, Madison, WI) was added and optical density (OD) readings were taken at a wavelength of 490nm every 30 min for 4 hours. The ODs were then analyzed against a calibration curve to quantify the number of
cells per well. The basis of this assay is that the quantity of Formazan produced from MTS by dehydrogenase in metabolically active cells is directly proportional to the number of living cells in culture (Promega).

2.7 Elispot Assay

The day prior to harvest, 96 well plates were coated with primary antibody for IFN-γ 2μg/ml (E. Bioscience) or IL-4 12μg/ml (generously provided by the Tary-Lehmann lab #11b11) and incubated overnight at 4°C. Prior to adding spleen cells, plates were washed with sterile PBS 3x200μl and blocked for an hour with sterile PBS containing 1%BSA and then again washed 3x200μl with sterile PBS. Unfractionated splenocytes, prepared as described above, were added at 3x10^5 cells/well final concentration with antigen for 48 hours in complete RPMI (Gibco with 1%P-S and 10%FBS). Cells were cultured in triplicate and stimulated with H. pylori lysate (12μg/ml final concentration, PHA (10μg/ml) or no stimulus. After incubation, plates were washed with 3x200μl PBS and then biotinylated secondary antibody against IFN-g 0.25μg/ml (generously provided by the Tary-Lehmann lab #R4682) and IL-4 0.25μg/ml (Pharmingen) were added and the plates were incubated overnight at 4°C. Plates were again washed 4x200μl PBS and AntiFITC-alkaline phosphatase (1:500, Molecular Probes) for IFN-γ and Strepavidin-HRP (1:2000 Dako) for IL-4 were added and incubated for 2 hours before adding developing solution. The reaction was then stopped by washing the plates with distilled water. Plates were then read on an Elispot reader (Cellular Technology Ltd., Cleveland, OH) to count quantity of spots, representing cells releasing that particular cytokine.
2.8 Histology and Inflammation Scores
A section of stomach taken, as described above, was prepared for histology (12). Briefly, the sections were formalin fixed and embedded in paraffin then sectioned at 5nm and stained with Hematoxylin and Eosin. All slides were scored by the same pathologist (RR) blinded to sample identity. Gastric inflammation was graded on a scale of 0 to 5 (12, 20)

2.9 Statistical Analysis
Data are represented as means for each group and statistical significance of differences between treatments was determined by T tests and ANOVA using Stat-View software v.4 (Abacus, Calabasas, CA) for the Macintosh. Box and whisker plots were used to determine any outliers, a maximum of one outlier could be removed from any one group and number reduced. Differences were considered significant if the p value was less than 0.05.

3. Results
3.1 LepR on T cells is Necessary for Clearing H. pylori Infection.
Our first goal was to utilize adoptive transfer of LepR negative T cells into H. pylori infected SCID mice in order to examine the role Leptin signaling on T cells plays in H. pylori clearance. This was accomplished by infecting SCID mice with H. pylori, then adoptively transferring T cells and quantifying the number of organisms by culture of standardized stomach homogenate at sacrifice. As seen in Figure 1, SCID mice that were challenged with H. pylori without adoptive transfer of T cells had prolific colonization of the stomach, with 6.79 ±0.43 log cfu H. pylori /g stomach (wet weight) (n=10). These mice showed no sign of being able to clear the infection. Recipient SCID
mice given adoptively transferred Leptin receptor positive (Lean) T cells showed significant clearance of *H. pylori*, with only $0.99 \pm 1.49$ log cfu/g stomach and 6/9 showing no sign of *H. pylori* in culture. In contrast, the mice given LepR\(^{-/-}\) T cells by adoptive transfer exhibited only partial clearance of the infection, with $3.52 \pm 2.85$ log cfu/g stomach with 3/9 showing no sign of *H. pylori* in culture. After finding differences in the ability to clear infection between mice adoptively transferred with LepR\(^{-/-}\) versus Lean CD4+ T cells, further investigation was necessary to probe the mechanisms involved by examining T cell proliferation and cytokine synthesis.

### 3.2 Absence of the Leptin Receptor Reduces Proliferation of Splenocytes In Response to *H. pylori* Antigen.

In order for an adoptive T cell response to occur, naïve T cells encounter antigen must first expand by proliferation and differentiate into effector T cells. A proliferation assay was used to determine if there were any differences in T cell response to *H. pylori* in the various groups of mice. When exposed to *H. pylori* lysate, splenocytes from animals given Lean T cells showed a significant amount of proliferation in comparison to the other two groups with an OD of 0.24, n=6 (p<0.008). SCID mice left without adoptive transfer mice exhibit minimal proliferation. Recipients of LepR\(^{-/-}\) T cells were again in the middle. Although a significant difference to SCID mice was not observed for recipients of Lepr\(^{-/-}\) T cells, a trend appears to be present (Fig. 2). A similar difference was seen between the LepR\(^{-/-}\) and Lean T cells when exposed to PHA mitogen (data not shown) indicating that the LepR\(^{-/-}\) T cells are diminished in their capability of proliferating at a similar rate to Lean (wt) T cells which is consistent with prior publications. In addition to the reduced capacity of LepR\(^{-/-}\) T cells to proliferate as
strongly as Lean cells in response to *H. pylori* antigen, cytokines released from LepR-/- T cells may also differ from Lean T cells when exposed to *H. pylori* antigen in vitro.

### 3.3 LepR⁻/⁻ T cells Elicit a Reduced Proinflammatory Cytokine Response in Comparison to Recipients of LepR Positive Cells

An inflammatory response has been established as a requirement for clearance of *H. pylori* infection. Cytokines released by lymphocytes affect the behavior of surrounding cells, and cytokines such as IFN-γ are associated with inflammation. An ELISPOT assay was performed to measure cytokine production from splenocytes incubated with *H. pylori* lysate to determine if Lepr⁻/- T cells provide as strong a signal for inflammation as Lean T cells produce. As seen in Fig. 3, animals adoptively transferred with Lean T cells show a large release of IFN-γ, a cytokine recognized to correspond with inflammation. While the LepR⁻⁻ recipients release half as much IFN-γ, they produce 4 times as much IL-4, an anti-inflammatory cytokine, in comparison to the Lean mice. Therefore, signaling through the LepR promotes an adaptive immune response that favors inflammation in infected SCID mice that have received Lean T cells whereas LepR⁻⁻ T cell recipient mice show an anti-inflammatory signal.

### 3.4 Inflammation in Infected SCID Mice After Adoptive Transfer.

To make sure that the cytokine signature corresponded to true inflammation levels, histological sections of the stomachs were scored for inflammation in each group. Histological sections of the stomachs were stained with H & E and inflammation was scored as described in the methods. As seen in Fig. 4, SCID mice with no adoptive transfer show minimal inflammation, whereas recipients of either LepR⁻⁻ or Lean T cells exhibited highly significant inflammation compared to SCID ((p<0.001 and 0.000006, respectively) but when compared to one another, similar degrees of inflammation were
seen (p= ns between the two recipient groups). This suggests that the connection between the cytokines and inflammation is a more subtle process.

**Discussion**

The data presented here indicate that the LepR on CD4+ T cells is required for complete clearance of *H. pylori* from infected SCID mice adoptively transferred with CD4+T cells. These results derive from the fact that only partial clearance of infection was observed in LepR−/− recipients. The well recognized reciprocal relationship between gastritis and bacterial load reported in many prior publications is repeated here (8, 9, 11, 12, 20). Furthermore, the more intense inflammation present in the recipients of Lean cells correlates with a “pro-inflammatory,” Th1 cytokine polarity. In contrast, the adaptive immunity conferred by LepR−/− T cells is oriented to a Th2 polarity, rich in IL-4 but weak in IFN-γ. Recipients of Lean T cells show greater gastric inflammation and further reduction of colonization relative to the LepR−/− recipients. Finally, these results suggest the novel hypothesis that the leptin receptor can shape the cytokine polarity of the adaptive immune response to *H. pylori*, and therefore alter both inflammation and clearance of infection.

Although leptin has been shown to exert innate anti *Helicobacter* response via cPLA2 activation and arachidonic acid release (32), what we have seen is due to adaptive T cell activation. The design of these experiments substantially restricts differences among the groups to CD4+ T cells and the adaptive immune response, and sharply curtails the influence of the innate immune response. Transfer of only a CD4+ subset narrows difference between the two adoptive transfer recipient groups to only the Leptin receptor on the CD4+ T cell. By using SCID mice in our model instead of Rag KO mice,
we were able to more closely relate gastritis to the T cells. Rag KO mice have been shown to develop more severe intestinal inflammation from the adoptive transfer process, regardless of infection, than SCIDs (25). In addition, the use of SCIDs has an advantage over nudes because SCIDs retain normal levels of macrophages, granulocytes, and NK cells (Jackson laboratories).

4.1 LepR on T cells is Necessary for Clearing H. pylori Infection.

Increasingly, signal transduction through the leptin receptor is recognized as a powerful modulator of the adaptive immune response to a wide range of immunogens, including various infectious pathogens (15, 18, 21). Signals which are mediated through the PI3 kinase pathway may be affected by the LepR. Leptin has been shown to reduce the effect of MAPK/ERK and PI3K inhibitors on LPS-induced apoptotic events (32). In addition to activating STAT1, 3, and 5 LepR can also induce PI3K and MAPK through MEK. This may be another way of stimulating a Th1 response. Diminished positive regulation of Th1 via MEK and PI3K may account for the more robust Th2 response in recipients of LepR^{-/-} T cells.

Our study shows that adoptive transfer of Lean naïve CD4^{+} T cells confers protection by creating a Th1 inflammatory response. Interestingly, the LepR^{-/-} T cells were able to partially clear the infection. Perhaps a time course study would better illustrate the response to infection. Immunized challenged mice show a fast and dramatic increase in inflammation and clear infection promptly. In contrast, unimmunized challenged C57BL/6 mice did not clear infection but developed more severe gastritis by 16 weeks post infection (12). Extending the time course in future studies may show that the LepR^{-/-} may require more time to build a more substantial response. This would be
consistent with a prior study that looked at delayed colitis after adoptive transfer of LepR\(^{-/-}\) T cells (31). In addition it may also be useful to look at earlier time points, because in an active immunization model of immune competent mice, inflammation was already beginning to subside at four weeks (12). This could also explain the lack of significance between the inflammation scores of Lean and LepR\(^{-/-}\) recipient mice.

In results similar to ours, but using active immunization instead of T cell adoptive transfer, vaccinated LepR\(^{-/-}\) mice were unable to clear infection while wt immunized mice did clear infection (34). These results corroborate our findings, but our work extends the results by demonstrating a critical role for the leptin receptor on T cells.

4.2 LepR\(^{-/-}\) T cells Exhibit Defective Clearance of H. pylori

There are several possible reasons why LepR\(^{-/-}\) T cells are poor at clearing infection. It is plausible that in our LepR\(^{-/-}\) adoptively transferred mice, an increase of T regulatory cells may have been able to better reduce effector T cells (7). DeRosa reported that use of ani-leptin antibody in culture with peripheral blood lymphocytes showed a dose dependent increase in Treg cell proliferation and reduction in effector T cell proliferation. In contrast, adding exogenous leptin had the opposite effect and reduced T reg proliferation and increased anergy of the T regs. We did not fractionate T cells into CD25- and CD25+ (regulatory T cells) when adoptively transferring, and it could be assumed that lack of the LepR on the T cells would have the same effect in the gastric mucosa and allow the T reg cells to restrict inflammation better in the in vivo LepR\(^{-/-}\) model.

Another reason LepR\(^{-/-}\) T cells were less successful could be due to an insufficient ability for the T cells to proliferate. Our data suggests a trend that shows LepR\(^{-/-}\) T cells are less able to proliferate in the presence of H. pylori antigen or phytohemaglutinin
(PHA) mitogen. This data is supported by prior findings that T cells lacking the leptin receptor are less able to respond. Though our culture was created from whole splenocytes that included antigen presenting cells (APC), to better demonstrate this additional APCs could be pulsed with *H. pylori* antigen and then co-cultured with the splenocytes to provide a more robust signal. Furthermore, a deviated cytokine pattern can be seen in the LepR-/- T cells as seen in Fig. 3. A Th2 pattern can be seen with elevated IL-4, possibly restricting the elevation of IFN-γ and subsequent inflammation. Finally, as discussed above and demonstrated in the Siegmund colitis model (31), slower kinetics of the inflammatory response induced by LepR-/- T cells could yield reduced clearance.

It is becoming ever more apparent that clearance of *H. pylori* requires inflammation driven by CD4+ T cells expressing the LepR. Continued work in this area to elucidate the exact mechanism whereby the Leptin receptors moderate inflammation may lead to better treatments for the disease.
Figure 1. Clearance of *H. pylori* four weeks post adoptive transfer of CD4+ T cells.
Bacterial cultures taken from the stomach show recipients of lean control (LepR+/+,-/+,-) T cells with established infection exhibited decreased colonization by 5 logs, nearly clearing infection entirely and were significantly lower than both unmanipulated SCID mice (p<0.000000001) and recipients of LepR-/- cells (p<0.03). Recipients of LepR deficient T cells were able to significantly (p<0.002) reduce the bacterial load in the stomach by more than 3 logs compared to unmanipulated challenged SCID mice. * Significant difference compared to all other groups.
Figure 2. Splenocyte proliferation after 48 hrs of *H. pylori* lysate stimulation. After a 48 hr culture with *H. pylori* antigen, the LepR-/- transplant recipients, though still able to recognize antigen with a value higher (OD=0.18, n=6) than that of the SCID mice (OD=0.14, n=3), were blunted in their ability to mount as strong a response as Lean controls. The Lean controls were able to respond to antigen and proliferate at a significantly greater level (p<.008, OD=0.24, n=6) than the other two groups. *Lean (wt T cells) significant compared to all other groups.*
Figure 3. Cytokine production after 24hr stimulation with *H. pylori* lysate. Though no significance is shown between the groups a trend appears to show that the Lean recipients had more of an inflammatory signature (high levels of IFN-γ and lower levels of IL-4) and the LepR<sup>−/−</sup> recipients show an opposite response more akin to a Th2 response (high levels of IL-4 and lower levels of IFN-γ).
**Figure 4** Inflammation is significantly higher in SCIDS with CD4+ T cell adoptive transfer. Histological sections were stained with H&E and then scored by a pathologist (RR) for gastritis. SCID mice with LepR/- and Lean CD4+ T cells adoptively transferred produced a significant (p< 0.05) level of inflammation within the stomach compared to SCID mice with no cell transfer. Mice which received LepR+ cells averaged a score of 2.17 which corresponds to a larger focus of inflammation extending between the glands and also reaching the submucosa. The mice which received Lean cells averaged a score of 2.61 and corresponding to inflammatory cells between glands and in the submucosa with metaplasia and mild to moderate epithelial cell hyperplasia. There is no significance found between either the groups that received CD4+ T cells.

*Significance to SCID mice without adoptive transfer.*
Bibliography


