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Enteral versus Parenteral Nutrition: Effect on Intestinal Barrier Function

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Abstract

Total parenteral nutrition (TPN), or the complete absence of enteral nutrients, is commonly used in a clinical setting. However, a major consequence of TPN administration is the development of mucosal atrophy and a loss of epithelial barrier function (EBF); and this loss may lead to an increase in clinical infections and septicemia. Our laboratory has investigated the mechanism of this TPN-associated loss of EBF using a mouse model. We have demonstrated that the mucosal lymphoid population significantly changes with TPN, and leads to a rise in IFN-γ and decline in IL-10 expression – both of which contribute to the loss of EBF. Associated with these cytokine changes is a dramatic decline in the expression of tight junction and adherens junction proteins. This article discusses the potential mechanisms responsible for these changes, and potential strategies to alleviate this loss in EBF.

Keywords

epithelial barrier function; tight junction; Intraepithelial lymphocyte; Interleukin-7 (IL-7); interferon gamma (IFN-γ); IL-10; Mouse; epithelial cells

Introduction

The intestinal epithelium undergoes both morphological and functional changes during total parenteral nutrition (TPN) administration. Many patients, because of either gastrointestinal dysfunction or a lack of intestinal length, are unable to tolerate enteral feedings. These patients are confined to a prolonged course of TPN. It is estimated that over 250,000 patients receive TPN in the United States alone on a yearly basis [1], resulting in over 11.5 million patient days of care per year. Increasingly, investigators have found that the intestine undergoes significant changes during the administration of TPN with an absence of enteral nutrition. In several TPN animal models, the intestinal epithelium shows a loss of villus height and a decline in epithelial growth [2, 3]. Further analysis of the mucosa shows a loss of epithelial barrier function (EBF) during the administration of TPN [4]. This loss of barrier function may result in endotoxins and even bacteria entering the systemic circulation.

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Clinical Consequences of a Loss of Epithelial Barrier Function with TPN

Loss of EBF with TPN may well be a potential mechanism for the observed higher rates of septicemia and other clinical infectious processes. Significantly higher rates of sepsis and infection have been strongly associated with TPN in the clinical settings of surgery and trauma [5, 6]. The etiology of these increased rates of infection is controversial. Systemic immunologic changes are quite common during the administration of TPN, and include both a decrease in absolute lymphocyte counts and a depression in lymphocyte function [7-9].

Use of a TPN Mouse Model to Study Mucosal Atrophy and Epithelial Barrier Function

The mouse model of TPN has yielded valuable insights, and may actually more closely resemble clinical TPN administration than other animal models. Previous studies with TPN in rat models showed a rapid progression of villus atrophy within a matter of 3 days [10]. Such changes do not occur as rapidly in humans receiving TPN, and thus must be carefully interpreted. Mouse models of TPN show the development of villus atrophy and formation of an epithelial barrier leak in a delayed period of onset (twice as long as rats), and more similar (although still more rapid) to humans.

TPN administration results in a loss of mucosal barrier function

Loss of EBF has been identified both in experimental models of TPN [3, 10, 11] and in humans [12]. The mechanism by which TPN leads to a loss of epithelial integrity has only recently been approached. *In vitro* cultures of intestinal epithelial cells (EC) have given critical insights into these mechanisms. A number of cytokines influence EBF. This was first shown in human intestinal EC (T84 cell line) incubated with interferon gamma (IFN-γ) [13]. Using a similar *in vitro* model, others have shown that pretreatment with transforming growth factor beta 1 (TGF-β1) can prevent the effects of IFN-γ [14]. A number of other cytokines have also been shown to affect EBF. Similar to TGF-β1, IL-10 can attenuate the loss of epithelial barrier in T84 monolayers caused by IFN-γ [15]. IL-4, however, can lead to a loss of EBF in a similar *in vitro* model [16]. Other cytokines which have been associated with a loss of EBF include IL-6 and tumor necrosis factor alpha (TNF-α) [17]. Loss of barrier function with TPN appears to be closely associated with the increased expression of IFN-γ by intraepithelial lymphocytes (IEL) [11, 18].

Gut-Associated Lymphoid Tissue (GALT)

The gastrointestinal tract is exposed to a number of foreign antigens on a daily basis, including toxins, bacteria, viruses and a number of diverse enteral nutrients. A critical part of protecting the host from these factors is the GALT. The GALT is a complex immunologic organ which contains several layers within the intestinal tract [19-21]. The layer of the GALT closest to the lumen is the intraepithelial lymphocytes (IEL). The IEL has a unique T-cell phenotype, and plays a critical role in both proliferative and cytotoxic T-lymphocyte actions [22, 23]. The IEL phenotype predominately consists of $CD4^-$,$CD8^+$ (70-85%, for mice, single positive), with other less frequent sub-populations: $CD4^-$,$CD8^-$ (5-10%, double negative); $CD4^+$,$CD8^-$ (5-12%, single positive); and $CD4^+$,$CD8^+$ (4-10%, double positive) [24, 25]. The IEL also has a large number of γδ-TCR^+ cells (30-70% compared to less than 2% in peripheral blood lymphocytes of mice) [26]. The IEL is thought to be derived from both a thymic-dependent and a thymic-independent source. Although still controversial [27], studies suggest that the IEL originate from both the intestinal tract as well as the thymus [27, 28]. $RAG2^{-/-}$ mice injected with bone marrow of nude mice failed to develop peripheral T-cells – but did develop a nearly normal IEL population which contained $CD8^+$ T-cells with both γδ– or αβ-TCR^+ phenotype

[29]. Although still controversial, the thymic-independent portion of the IEL is best defined by the CD8αα homodimeric T-cell population. In contrast, CD8αβ (heterodimeric) cells are believed to be of thymic origin. The function of the IEL has not been completely determined. Using a redirected cytotoxic functional assay without *in vitro* activation, the IEL shows considerable activity [30]. The actual significance of these actions has not been completely defined, but suggests a role in protecting the organism from harmful intraluminal pathogens. The IEL is also a rich source of cytokines, including: IFN-γ [31], IL-2 [32], IL-4 [33], IL-6 [34], TNF-α [35], and TGF-β_1 [36]. TGF-β_1 has been detected in both αβ-TCR$^+$ and γδ–TCR$^+$ IEL subsets [37]. IL-10 has recently been shown to be expressed in the IEL, and may closely relate to the modulation and function of T-regulatory cells [38]. Cytokine production in the αβ-TCR$^+$ fraction of the IEL has been extensively studied, and the CD4$^+$,CD8$^-$ population expresses IFN-γ, IL-4 and IL-5 [37, 39]. The CD4$^+$,CD8$^+$ population expresses greater amounts of IFN-γ and IL-5. The CD4$^-$,CD8$^+$ population produces IFN-γ, IL-5 and some IL-6. It appears that a major aspect of IEL regulatory function is achieved by specific cytokine production. It is hypothesized that IEL cytokine production leads to modulation in epithelial cell function [40], as well as to a down-regulation of immunologic sensitization to foreign antigen [33].

IEL Closely Interact with Mucosal Epithelial Cells

Because of the close physical association of the IEL with mucosal EC, interactions between the two populations have been examined. EC express essential ligands which are needed for IEL to adhere and reside within the mucosal epithelium [41]. Interestingly, these ligands are expressed along the basolateral surface of the EC, in juxtaposition to IEL. Perhaps the best characterized of these is the integrin $\alpha_E\beta_7$ on the IEL and E-cadherin on the surface of the epithelial cells [42]. Another important manner in which the IEL and EC intercommunicate is via the large number of cytokines, growth factors and chemokines which are expressed by EC. One such example is that EC-derived IL-7 strongly influences the development, growth and phenotype of the adjacent IEL [43]. IL-7$^{-/-}$ or IL-7R$^{-/-}$ mice show distinct declines in absolute numbers of thymocytes and IEL, with a virtual absence of the γδ– TCR$^+$ population, and fewer αβ-TCR$^+$ T cells than wild-type mice [44].

Changes in the IEL with TPN Administration

Phenotypic changes

A number of IEL phenotypic alterations occur with TPN, and include a marked decline in the CD4$^+$, CD44$^+$ and CD8αβ$^+$ populations (Table 1). The CD4$^+$ population is known to be very responsive to exogenous stimulation, and its loss may explain an observed loss of IEL proliferative responsiveness with TPN [31]. The loss of the CD8αβ$^+$ population with TPN may represent a loss of the thymic-dependent population of the IEL [29], and the loss of CD44$^+$ cells suggests a shift to a less mature IEL [45]. Interestingly, the alteration in IEL phenotype did not correlate with enteral anaerobic or aerobic bacterial cultures, in that the number and strain of these bacteria did not significantly change with TPN [31]. An additional group is shown in Table 1, that of TPN mice allowed small amounts of enteral food (TPN+Food group; comprising 25% of caloric needs). Interestingly, IEL phenotype changes were significantly influenced by the loss of enteral nutrition; most of the observed IEL changes with TPN were reversed with the addition of this small amount of enteral nutrition. The mechanism by which enteral food prevents the observed IEL changes has not been determined.

Cytokine expression

Table 2 shows changes in cytokine expression with TPN [31, 36, 46]. This panel of cytokines was chosen because each of these has been shown to alter either barrier function or apoptotic signaling in the intestine [15, 16, 47-51]. Our laboratory reported a rise in IFN-γ expression with

TPN, and this could well impact both the observed increase of EC apoptosis [52] as well as a loss of EBF (see below). In addition to IFN-γ, other cytokines may also have relevance to EC physiology, including IL-4, IL-6 and IL-10 (Table 2). As mentioned above, each of these cytokines can contribute to changes in EBF. It is possible that the decline in IL-10 mRNA expression and the rise in IL-4 and IL-6 may all contribute to the development of the TPN-associated epithelial leak and increased rates in EC apoptosis. The administration of 25% enteral nutrition to TPN mice prevented these cytokine changes. As mentioned earlier, EC-derived IL-7 is another important cytokine within the intestinal mucosa. Our laboratory has shown a close physical relation between EC which express IL-7 and IEL [53]. Subsequently, we showed that the administration of TPN results in a significant decline in IL-7 expression [54]. With the administration of exogenous IL-7, much of the IEL phenotypic changes associated with TPN can be prevented [54], suggesting that IL-7 may have an important role in the mediation of the changes to the mucosa with TPN administration.

Impact of IEL-derived Cytokines and Epithelial Barrier Function

TPN-associated increases in IEL-derived IFN-γ and decline in IL-10 expression have both been associated with a loss of EBF [11, 18, 55]. A significant decline in transepithelial resistance (TER) of full-thickness ileum is noted in the TPN group. In order to determine the influence that increased expression of IFN-γ had on this change in EBF, a series of IFN-γ knockout mice underwent TPN administration. In the absence of IFN-γ, the degree of TPN-associated epithelial barrier breakdown, as measured by either the permeability coefficient (Papp) or by transepithelial resistance (TER) (Figure 1), was significantly lessened [46]. The fact that permeability levels did not completely return to normal suggested that other mediators of this breakdown exist. Whether this is a direct action of IFN-γ on the EBF, or if acts to prime tumor necrosis factor receptors remains to be determined [56]. Subsequently, our group then determined the influence of the decline in IL-10 with TPN administration. Sun, et al demonstrated that the additional administration of exogenous IL-10 to mice receiving TPN resulted in a return of EBF toward normal [55]. Similar to the IFN-γ mice, exogenous IL-10 partially prevented this barrier loss; however, the effect was only about 50% effective. This suggests that the cytokine changes with TPN administration account for a substantial degree of EBF loss.

Alteration in the Tight Junction with TPN

The etiology of this loss of EBF was further investigated. Our laboratory detected a substantial decline in the expression of a number of tight junction molecules [55], including ZO-1, occluden, several claudins and JAM-1, as well as the adherens molecule E-cadherin. Figure 2 shows immunofluorescent images of some of these factors. In addition to a loss of expression, internalization (endocytosis) of some of these factors can be seen – a finding similarly observed *in vitro* with cytokine exposure to epithelial monolayers, as well as other clinical disease processes where EBF is lost such as in inflammatory bowel disease [50, 57, 58].

Therapeutic Options to Prevent Barrier Loss with TPN

The IEL not only expresses a number of cytokines, but the γδ-TCR sub-population has been shown to express keratinocyte growth factor (KGF, or fibroblast growth factor-7) [59]. Our group has subsequently demonstrated that with TPN administration, there is a marked decline in KGF expression by this IEL sub-population [60]. Further, the exogenous administration of KGF to mice receiving TPN was able to significantly prevent loss of villus height, crypt depth and restored EBF (Figure 3) [61]. This suggests that the use of KGF may have a therapeutic potential for patients on long-term TPN, and may prevent many of the complications associated with EBF loss.

Conclusions

TPN results in a loss of villus height, loss of EC proliferation, rise in EC apoptosis, and a marked decline in EBF. Using a mouse model of TPN, the IEL has been shown to undergo significant changes. Such changes may partially account for the mechanism of EBF loss with TPN, and may contribute to the increased incidence of septicemia with TPN administration. The mechanisms by which IEL change with TPN are not known, but data suggest that such changes are due to a lack of enteral nutrition and may be mediated by a decline in EC-derived IL-7. Understanding the mechanisms which are responsible for such changes may lead to the potential for therapeutic options in patients receiving TPN. Further, understanding the changes in the IEL with TPN administration may also yield important information in how EC-IEL interactions take place. Such interactions appear to directly influence observed alterations in the physiology of the intestinal mucosa. Thus, these cell-cell interactions may have important applications in other processes such as gut adaptation after bowel resection, or during autoimmune processes such as inflammatory bowel disease.

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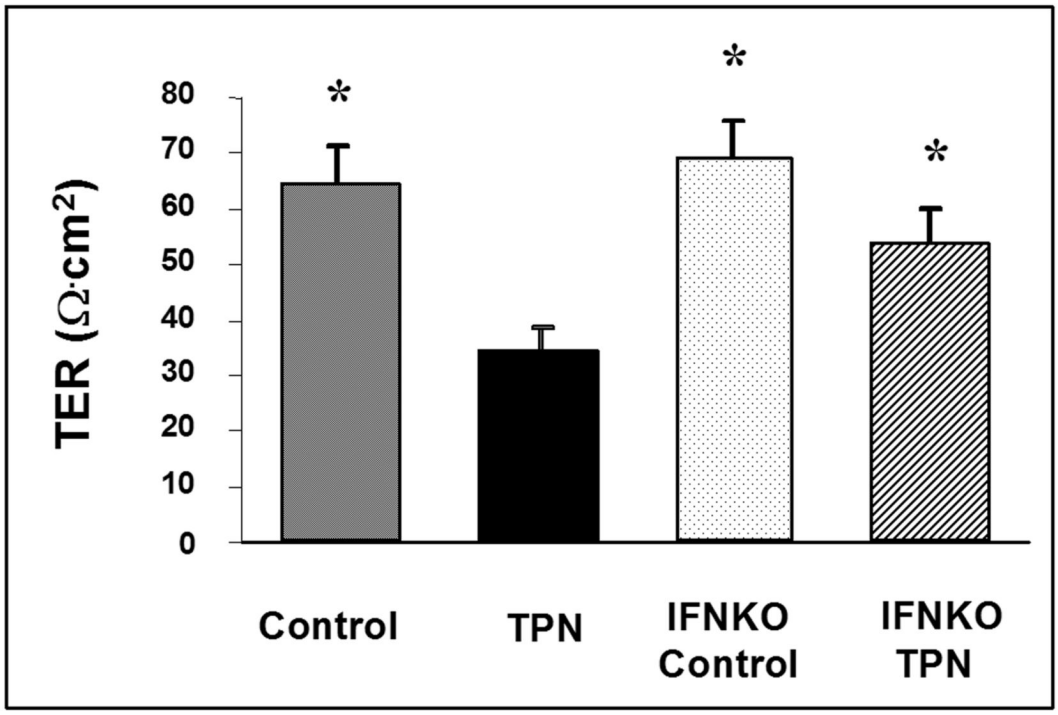

Figure 1.

Transepithelial resistance (TER) in mice was measured in Ussing chambers after a 30 min equilibration period. Results (mean ± SD) in 6 mice per group. TPN significantly decreased intestinal TER in wild-type mice. Elimination of IFN-γ was achieved using knockout mice (IFNKO). Significance was found between the TPN and all other study groups; the other groups were not significantly different between themselves. ANOVA was used for statistical analysis. **P*<0.05 comparing TPN group to other groups, with a Bonferonni post hoc analysis.

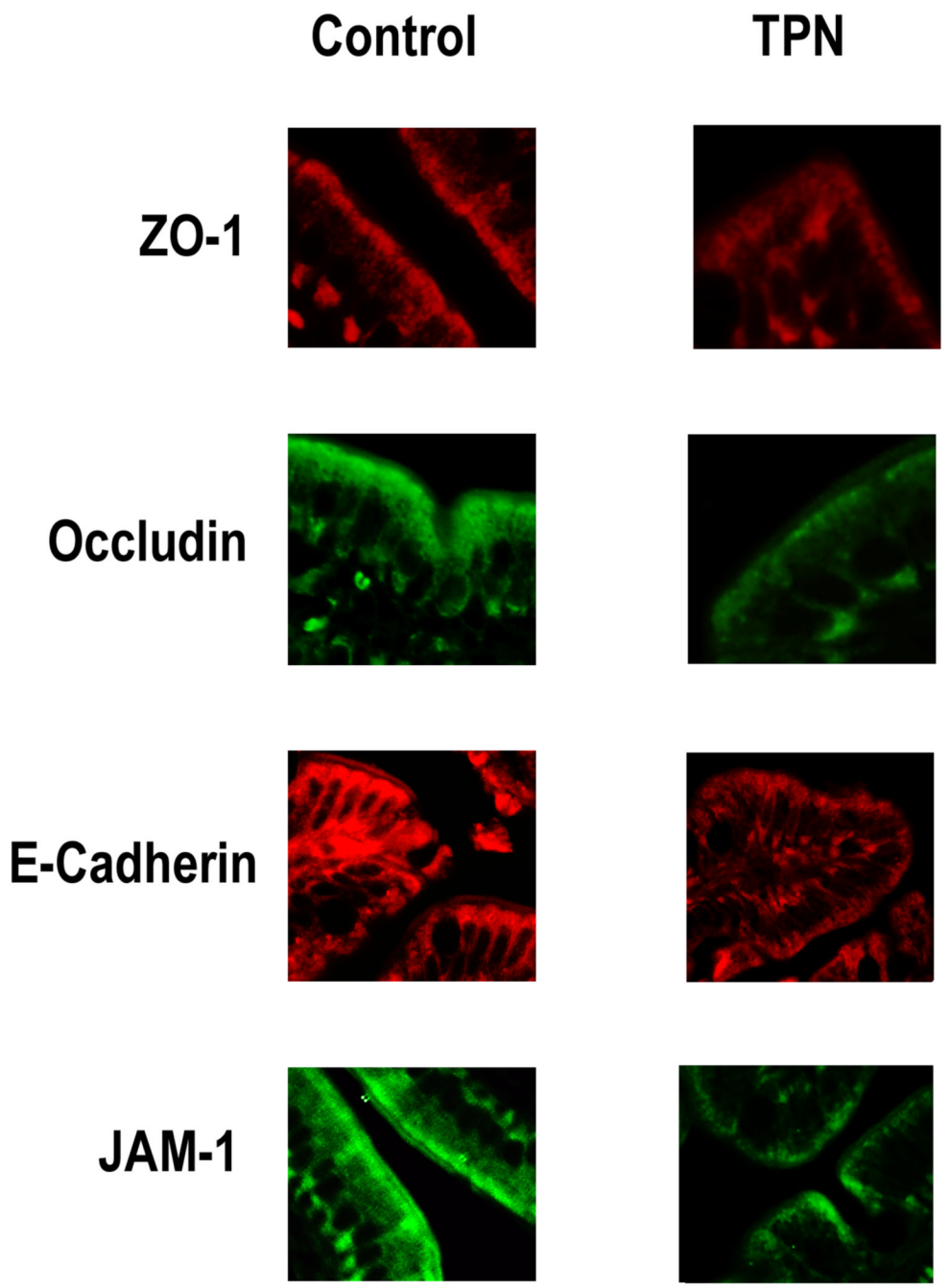

Figure 2.
Immunofluorescent images of representative portions of mid-small bowel mucosa in Control (enterally fed) and TPN study groups. Note the loss of junctional and adherens proteins with TPN. Additionally, note the movement of some of these proteins (occludens) into the cytoplasm with TPN administration.

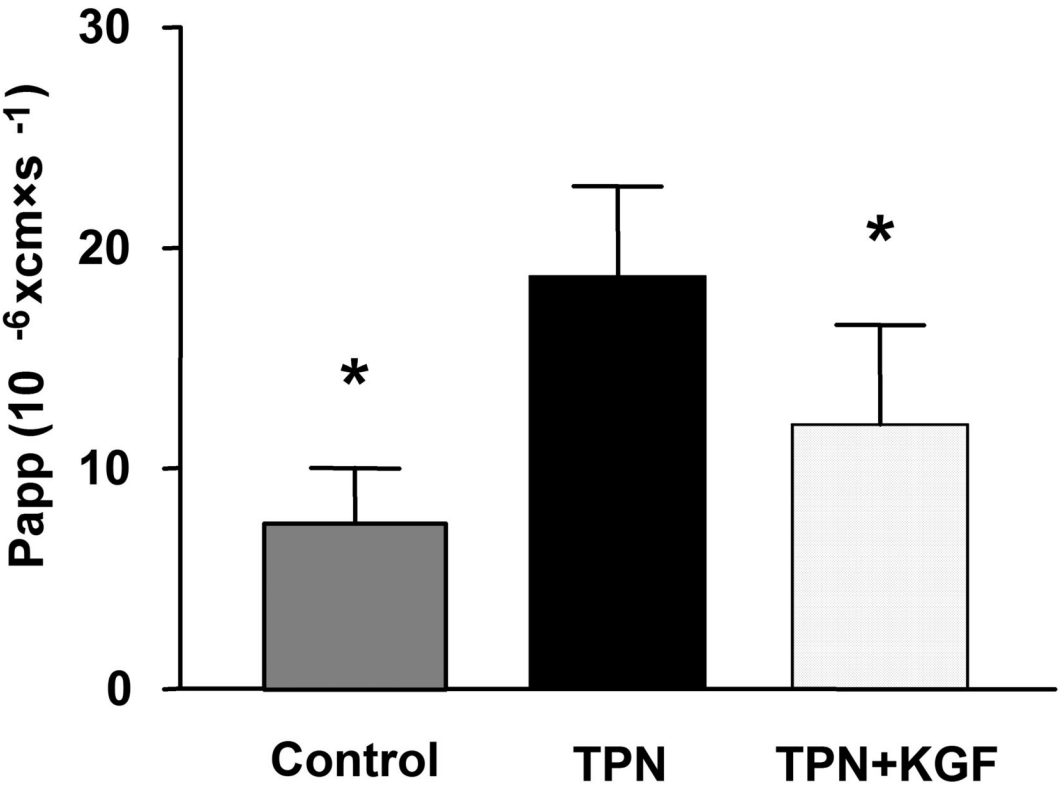

Figure 3.
Effect of TPN on the permeability coefficient (Papp) of tritiated mannitol as measure in Ussing chambers of full-thickness ileal specimens with TPN administration (*P<0.05). Note also the return of permeability to control levels when TPN mice are given exogenous keratinocyte growth factor (KGF) (5mg/kg/day) [62].

Table 1

Small bowel IEL phenotype in Control and TPN mice. Column on right are mice maintained on TPN and allowed *ad lib* chow. Results are from flow cytometry studies after gating on the IEL population. CD4,CD44 results not shown because of the marked loss of the $CD4^+$ population with TPN. Results are expressed as mean % gated IEL (ranges) and represents an N=6 mice for each group.

IEL Phenotypes	**Description**	**Control (range)**	**TPN (range)**	**TPN+Food (range)**
CD4 and CD8α				
$CD4^+CD8^-$	single positive	**4.7** (3.9-6.1)	**0.6** (0.2-0.7)*	**2.5** (1.9-2.8)
$CD4^+CD8^+$	double positive	**2.8** (2.5-4.3)	**0.6** (0.1-1)*	**5.4** (3.8-12.1)
$CD4^-CD8^-$	double negative	**28** (23-34)	**35** (19-64)	**16** (12-28)
$CD4^-CD8^+$	single positive	**65** (58-70)	**64** (37-79)	**72** (67-77)
CD8αα and CD8αβ				
$CD8\alpha\alpha^+$	thymic-independent	**64** (59-71)	**52** (40-63)	**72** (66-78)
$CD8\alpha\beta^+$	thymic-dependent	**4.8** (3.0-6.5)	**0.4** (0.1-0.8)*	**7.4** (5.6-8.6)
TCR-αβ vs. TCR-γδ				
$TCR\text{-}\alpha\beta^+$	T-cell receptor	**39** (33-43)	**22** (21-31)	**24** (18-28)
$TCR\text{-}\gamma\delta^+$	T-cell receptor	**62** (53-68)	**70** (57-76)	**57** (51-62)
CD44	T-cell maturity			
$CD8^+,CD44^+$		**29** (28-37)	**10** (1.0-18)*	**40** (38-46)

*P<0.01, by ANOVA.

Table 2

mRNA expression of IEL cytokines in control, TPN and TPN+Food groups. Note the significant alterations in all measured cytokines with administration of TPN, and the prevention of these changes with the addition of enteral feedings to TPN mice. N=6,

Groups	IL-4	IL-6	IL-10	IFN-γ
Control	0.10±0.1	0.02±0.0	0.55±0.2	0.15±0.1
TPN	0.73±0.3**	0.95±0.3**	0.23±0.1**	0.29±0.1*
TPN+Food	0.11±0.1	0.05±0.0	0.65±0.2	0.14±0.0

* *P* <0.05,

** *P*<0.01 TPN vs. Control and TPN+Food; using ANOVA. In general a Bonferonni post hoc t test is used for remaining Preliminary results. Results are the mean (±SD) mRNA expression of cytokine from purified IEL (via magnetic beads) samples, and expressed as the ratio of cytokine expression to beta actin. Abbreviations: IFN-γ: interferon gamma. (Data submitted to J Surg Res).