Colonic Epithelial Physiology Is Altered in Response to the Bacterial Superantigen Yersinia pseudotuberculosis Mitogen

G. A. E. Donnelly,1 J. Lu,1 T. Takeda,2 and D. M. McKay1

1Intestinal Disease Research Programme, McMaster University, Hamilton, Canada; 2Department of Infectious Diseases Research, National Children’s Medical Research Center, Tokyo, Japan

Because bacteria are implicated in the pathophysiology of gut inflammation, the ability of the superantigen Yersinia pseudotuberculosis mitogen (YPM) to alter epithelial ion transport and permeability was examined by two model systems: epithelial (T84) monolayers cocultured with peripheral blood mononuclear cells (PBMC) with or without YPM and colonic segments from YPM-treated mice. YPM immune activation in vitro caused reduced active ion transport responses to the prosecretory agent forskolin (increases cAMP) and increased permeability. Similar changes in T84 function were evoked by conditioned medium (CM) from YPM-activated PBMC, and tumor necrosis factor–α and interferon–γ were mediators of these events. Inclusion of piroxicam in the CM prevented increases in epithelial permeability but did not ameliorate the perturbed ion transport. Colonic tissue from YPM-treated mice displayed diminished responsiveness to cAMP-mediated secretagogues and nerve stimulation. Thus, Y. pseudotuberculosis enteric symptomatology may be at least partially due to YPM, and superantigens have the potential to initiate or exacerbate gut dysfunction.

Infections with the gram-negative enteropathogenic bacillus Yersinia can result in intestinal discomfort, variable diarrhea, inflammation in the terminal ileum, and systemic symptomatology such as fever, rash, and lymphadenopathy [1]. Bacterial superantigens (SAgs) are small peptide molecules that activate large numbers of T cells (≤25%) by cross-linking major histocompatibility class II antigens with the appropriate domain of the variable portion of the β chain (Vβ) of the T cell receptor [2]. A novel SAg designated Yersinia pseudotuberculosis mitogen (YPM) has been isolated [3] and implicated as an important molecule in the pathogenesis of Y. pseudotuberculosis infection [1, 4]. Bacterial SAgs of unknown origin have been implicated in autoimmune and inflammatory disease [5], including inflammatory bowel disease (e.g., Crohn’s disease) [6]. It is noteworthy that Y. pseudotuberculosis isolated from animals with systemic infections all produced YPM and that many of the strains that caused gastroenteric-type infections also produced YPM [7].

There has been significant progress in unraveling the structure and immune-activating properties of YPM [8]; however, there is a lack of information on the physiologic consequences of exposure to this bacterial SAg, particularly in terms of gut function. In the present study, in vitro and in vivo strategies were used to examine the effect of YPM on enteric epithelial ion transport and permeability.

Materials and Methods

Cell Culture Studies

YPM immune activation. Peripheral blood mononuclear cells (PBMC; 106 cells/well) were isolated from healthy volunteers [9] and incubated at 37°C for 24 h with or without YPM (100 ng/well; lipopolysaccharide-free) in 96-well plates. Each well was pulsed with 1 μCi of [3H]thymidine (DuPont, Wilmington, DE), cells were harvested, and radioactivity was determined in a scintillation counter (Becton Dickinson, Mississauga, Canada) 18 h later.

To determine cytokine production, 106/mL PBMC were cultured with or without YPM (1 μg/mL) for 24 h, and tumor necrosis factor (TNF)–α (Biotrack, Oakville, Canada), interleukin (IL)–2 (Advanced Magnetics, Cambridge, MA), and interferon (IFN)–γ (Sero-Tech, Toronto) production was determined by ELISA. Cytokine assays were conducted in duplicate serial dilutions and had a detection limit of 4 pg/mL. Prostaglandin E2 (PGE2) levels were measured by EIA (Amersham Pharmacia Biotech, Piscataway, NJ; sensitivity, 50 pg/mL) in conditioned medium (CM) from YPM-activated PBMC before and after exposure to T84 cells (human colonic crypt–like epithelial cell line). Also, T84 cells (2 × 104/well) were cultured for 7 days, treated with CM from YPM-activated PBMC for 24 h, rinsed twice, lysed per the manufacturer’s instructions (Amersham), and released (i.e., intracellular) PGE2 was measured.
Epithelial ion transport and permeability. Coculture studies were done as described elsewhere [9]. In brief, filter-grown confluent monolayers (transepithelial resistance $\geq 1000$ $\Omega$ cm$^2$; determined by choppstick electrodes and a voltmeter [Millicel-ERS; Millipore, Bedford, MA]) were cocultured for 24 h with PBMC (10$^6$ cells [10]) with or without YPM (1 ng/ml $\mu$g/mL). Immune cells with or without YPM were added to the basal compartment of the coculture well. In some experiments, PBMC and YPM were concomitantly treated with piroxicam (10$^{-6}$ M; Sigma, St. Louis).

In additional experiments, PBMC were activated for 24 h with YPM (100 ng/mL), and cell-free CM was collected and diluted 1:1 in fresh media (50% CM) and then added with or without piroxicam (some studies used ketoprofen [10$^{-6}$ M; Sigma]) to the basolateral surface of the monolayers, and the maximum change in ISC was recorded [11]. Epithelial permeability was assessed by measuring transepithelial ion resistance [10]. Changes in net active ion transport were recorded in response to (1) electrical transmural field stimulation (ETS; at 10 Hz, 10 mA, 0.5 ms for 5 s total) and (2) addition of forskolin or PGE$_2$ (both 10$^{-5}$ M) to the buffer bathing the serosal side of the tissues [12].

Histology. A segment of proximal colon (taken at the ileocecal junction) was fixed in 10% neutral buffered formalin, dehydrated, and embedded in paraffin wax, and 3-$\mu$m sections were cut and stained with hematoxylin-eosin. Specimens were collected on coded slides and examined in a blinded fashion for evidence of damage, epithelial changes, and inflammatory infiltrates. Crypt depth was measured by using an eyepiece graticule on whole crypts defined by an intact epithelial layer.

Analysis

Data are expressed as mean ± SE. Because a degree of intersubject variability in T84 responses has been noted [9, 14], data were normalized to time-matched media-only treated control monolayers and are presented as percentage of control values. “$N$” values are the number of experiments (i.e., 2–4 T84 monolayers/experiment) or number of mice (2 colonic segments/mouse). We used Student’s t test to compare two groups and a one-way analysis of variance followed by post hoc comparisons with the Newman-Keuls test for multiple group comparisons. $P < .05$ was considered statistically significant.

Results

YPM in vitro and in vivo immune activation. In vitro exposure of human PBMC to YPM resulted in immune activation as assessed by cytokine production and a restimulation proliferation assay (table 1). The increased production of IL-2 indicated $T$ cell activation and confirmed previous observations [5]. Similarly, mice treated 4 h earlier with YPM had elevated serum levels of IL-2, and spleen cells isolated from these mice had enhanced proliferative responses to in vitro rechallenge with YPM compared with cells from time-matched control mice (table 1). Moreover, spleen cells from YPM-treated mice displayed greater spontaneous proliferation (5911 ± 1757 vs. 523 ± 86 cpm) and were more responsive to in vitro challenge with the mitogen ConA (50,613 ± 8012 vs. 23,530 ± 6793 cpm) and the unrelated SAg, SEB (12,847 ± 3355 vs. 1161 ± 347 cpm; mean ± SE; $n = 5$; $P < .05$ vs. control for all) as gauged by [H]$\text{H}$thymidine incorporation.

In vitro modulation of epithelial function. Baseline ISC was not significantly altered by exposure to YPM only, nonactivated PBMC, or YPM-activated PBMC (data not shown). Similarly, YPM only or PBMC only coculture had no or minimal effects on transepithelial resistance and the ISC response evoked by
forskolin. In subsequent experiments, we used T84s grown in medium only as controls.

In contrast to baseline ISC, change in ISC to forskolin was significantly reduced in T84 monolayers cocultured with YPM-activated PBMC for 24 h (figure 1). Concomitantly with this was a dose-dependent drop in transepithelial resistance (figure 1). These changes in epithelial function were due to a soluble mediator, since virtually identical epithelial irregularities were evoked by CM from the YPM-activated PBMC (figure 2). Addition of TNF-α antibodies to the CM led to a partial inhibition in the drop in transepithelial resistance, and the change in ISC to forskolin remained reduced (table 2). However, the combination of antibodies to TNF-α and IFN-γ completely prevented the CM (n = 3) from YPM-activated PBMC to alter T84 resistance and active ion transport (table 2).

Since prostaglandins directly affect epithelial function and can modulate cytokine synthesis [15], we examined the role of known inhibitors of prostaglandin synthesis in YPM + PBMC modulation of the epithelium. While piroxicam alone had no direct effect on T84 function (figure 3), it prevented the YPM + PBMC–induced drop in transepithelial resistance but had no beneficial effects in preventing the reduced change in ISC response to forskolin (figure 3). Piroxicam treatment also prevented the drop in resistance caused by exposure to CM, showing that the epithelium was the target cell for the piroxicam effect (control, 1153 ± 113; 24-h exposure to CM, 526 ± 90 [P < .05 vs. control]; CM + piroxicam, 1130 ± 250 Ω cm²; n = 6 monolayers). In comparison with the coculture experiments, exposure to YPM + PBMC-CM for 24 h resulted in a drop of ~30% in T84 responsiveness to forskolin that was not affected by coadministration of piroxicam: control, 131.8 ± 5.9; CM, 86.3 ± 3.2; and CM + piroxicam, 87.1 ± 5.9 μA/cm² (P < .05 vs. control). Similarly, ketoprofen treatment alone affected neither T84 transepithelial resistance nor change in ISC to forskolin, while it partially and significantly prevented the YPM + PBMC-CM–induced drop in T84 resistance (figure 4); ketoprofen did not affect the diminished change in ISC response to forskolin. Moreover, naive monolayers treated with arachidonic acid (10^{-6} M) and examined 24 h later in Ussing chambers did not display any alterations in resistance (control, 1309 ± 73; arachidonic acid–treated, 1104 ± 138 Ω cm²; n = 6 monolayers; P = .22) or secretory responsiveness to forskolin, which was 96.5% of control values.

PGE_2 was not detected in CM from YPM-activated PBMC before addition to T84 epithilia or on retrieval after 24 h of exposure to confluent T84 monolayers (n = 3). Also, intracellular PGE_2 was not increased in T84 cells treated with YPM-CM (data not shown).

**Epithelial integrity:** The ability of epithelial monolayers to

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**Figure 1.** Changes in transepithelial resistance (TER) and injected short-circuit current response to forskolin (Isc to FSK [10^{-7} M]) after 24 h of coculture with 10^6 peripheral blood mononuclear cells (PBMC) with or without *Yersinia pseudotuberculosis* mitogen (YPM). n = 3–5 experiments; letters indicate statistically similar groups and significant difference from control at P < .05; control resistance range, 1818–3333 Ω cm²; control FSK response range, 50–131 μA/cm².

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**Table 1.** Immune activation by *Yersinia pseudotuberculosis* mitogen (YPM).

<table>
<thead>
<tr>
<th>Effects of YPM</th>
<th>Nonactivated PBMC</th>
<th>YPM (1 μg/mL)-activated PBMC</th>
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<tbody>
<tr>
<td>In vitro (n = 3)</td>
<td></td>
<td></td>
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<tr>
<td>Proliferation, [^3H]thymidine incorporation (cpm)</td>
<td>260 ± 36</td>
<td>9744 ± 1282^a</td>
</tr>
<tr>
<td>Cytokine production (pg/mL)</td>
<td>&lt;4</td>
<td>257 ± 42^a</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>45 ± 30</td>
<td>1311 ± 175^a</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>39 ± 18</td>
<td>914 ± 112^a</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (non-YPM-treated mice)</td>
<td></td>
<td>Mice given YPM 4 h previously</td>
</tr>
<tr>
<td>Proliferation (spleen cells)</td>
<td>2031 ± 598</td>
<td>9911 ± 3035^a</td>
</tr>
<tr>
<td>[^3H]thymidine incorporation (cpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokine production (serum levels)</td>
<td>Not detected</td>
<td>11.7 ± 2.6</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Data are mean ± SE; PBMC, peripheral blood mononuclear cells.

* P < .05 vs. control.
vectorially transport charged ions, albeit at a reduced rate, indicates a functional monolayer. Determination of released LDH levels showed no significant increase in T84 cell cultures exposed to YPM-activated PBMC-CM compared to T84 cell monolayers grown in culture medium only (113.5 ± 4.5 and 111.5 ± 2.0 U/L, respectively).

Effect of YPM on murine colonic function and morphology. Analysis of colonic tissues from YPM-treated mice revealed an increase in baseline ISC (74 ± 8 vs. 43 ± 9 μA/cm²; n = 5; P = .026 vs. control), while conductance was unaffected by the experimental treatment: G = 20.5 ± 1.7 and 18.0 ± 0.9 mS/cm² for treated and control mice, respectively (t test, P = .216). In contrast, stimulated ISC responses to both ETS and PGE₂ were significantly diminished in colonic segments from YPM-treated animals (figure 5). Neuronally and cAMP-mediated changes in ISC responses were also reduced in colonic tissues of mice treated 4 h previously with SEB: ETS, 31 ± 4 versus 17 ± 3; forskolin, 123 ± 16 versus 53 ± 10 μA/cm² for control and SEB-treated mice, respectively (mean ± SE; n = 6; P < .05 vs. control). Colonic histology of mice treated 4 h previously with YPM or SEB was unremarkable. There were no signs of epithelial damage, edema, altered crypt architecture or size, or inflammatory infiltrate compared with colonic tissue from time-matched control mice.

Discussion

Skewing of T cell receptor Vβ expression in some persons with Crohn’s disease has led to the suggestion that SAgs are involved in human enteric inflammatory disease [6]. Indeed, 35 years ago the same postulate was expounded, and elegant descriptive studies illustrated the enteric histopathology evoked by treatment with crude preparations of S. aureus or SEB [16, 17]. These early studies did not examine gut function, although it was noted that the treated animals developed a variable diarrhea. Consequently, we have begun to explore the enteric physiologic consequences of exposure to bacterial SAgs, and the data presented here illustrate the ability of these potent immune stimuli to evoke altered epithelial permeability and/or ion transport.

Epithelial ion transport (the driving force for directed water movement) and barrier functions are important elements of host innate immunity that are typically perturbed in animal models of gut disease and in persons with a variety of idiopathic and T cell-driven enteropathies [18]. Y. pseudotuberculosis infection can result in gut disturbances [19, 20], and YPM is a probable pathogenicity factor in this infection [21]. Before assessing YPM’s ability to affect gut epithelial function, it was important to determine whether YPM elicited an immune response in the model systems used here. The increases in PBMC proliferation and cytokine production (including T cell-specific IL-2) confirmed the immunostimulatory properties of YPM [4]. Also, YPM was active in vivo, as judged by a hyperresponsiveness of splenocytes to subsequent in vitro YPM challenge. The activity status of gut-derived T cells was not examined in this study, although we have reported that like splenocytes, jejunal lamina propria lymphocytes from mice treated 4 h earlier with SEB are hyperresponsive to in vitro rechallenge with SEB [12].

Exposure of T84 epithelial monolayers to YPM-activated PBMC or CM from YPM-activated PBMC led to increased epithelial permeability and a reduced capacity to respond to forskolin. These data corroborate similar observations with the unrelated SAg, SEB [9, 10], and add further credence to the hypothesis that exposure to bacterial SAgs can result in perturbed epithelial physiology. The altered epithelial function was

Figure 2. Changes in transepithelial resistance (TER) and injected short-circuit current response to forskolin (Isc to FSK 10⁻⁴ M) after exposure to conditioned medium (50%) from peripheral blood mononuclear cells (PBMC) activated with Yersinia pseudotuberculosis mitogen (YPM; 100 ng, 24 h; n = 3–5 experiments; letters indicate statistically similar groups and significant difference from control at P < .05; control resistance range, 3100–2500 Ωcm²; control FSK response range, 51–151 μA/cm²).

Figure 3. Cotreatment with piroxicam (Pir; 10⁻³ M) prevents decrease in transepithelial resistance but not diminished short-circuit current response to forskolin (10⁻³ M) evoked by coculture with Yersinia pseudotuberculosis mitogen (YPM; 100 ng/mL)-activated peripheral blood mononuclear cells (PBMC) for 24 h (n = 3 experiments [6 monolayers]); *, P < .05 vs. control; control resistance range, 2000–3125 Ωcm²; control FSK response range, 31–97 μA/cm²).
accompanied by increased levels of TNF-α and IFN-γ, both of which can directly and indirectly modulate epithelial physiology [22]. The findings that neutralization of TNF-α and IFN-γ completely prevented YPM-CM from affecting T84 function is in accordance with data examining the mechanism of SEB modulation of epithelial function in vitro [10]. In addition, IFN-γ and TNF-α have been implicated in the pathophysiology of YPM and SEB toxic shock in mice [21, 23].

In addition to their ability to influence immune cell proliferation and cytokine production [15, 24], prostaglandins can be a final mediator in cytokine effects on epithelial function [25, 26], and enterocytes can respond to cytokines by increasing arachidonic acid metabolism [27]. Generally, prostaglandins are cytoprotective in the gut, mediating epithelial restitution [28, 29] and maintaining epithelial barrier function [30]. In contrast, piroxicam inhibition of prostaglandin synthesis correlates with enhanced barrier function in rodent gastric mucosa [31]. The effects of prostaglandins can also be modulated by the presence of other mediators [32].

Inclusion of piroxicam or ketoprofen (nonselective inhibitors of constitutive and inducible cyclooxygenase [COX I and II]) in epithelial PBMC + YPM cocultures or addition to CM from YPM-activated PBMC prevented the disrupted epithelial permeability but did not affect the diminished intracellular ion transport responses to forskolin. These data indicate that the enterocyte was the target for the drug activity. Arachidonic acid did not elicit any functional changes in naive T84 monolayers, suggesting that the immune mediators and not merely the prostaglandin precursor alone are required to evoke increased epithelial permeability. Moreover, T84 cells do not appear to express the inducible COX II (unpublished data), suggesting that the piroxicam effect was via modulation of COX I activity. Measurement of PGE2, as a representative prostaglandin that displays a loss of barrier function that was partially prevented by 5-aminosalicylic acid [34]; the investigators suggested that the drug competed for the IFN-γ receptor. Because the ability of piroxicam to abrogate a loss of epithelial barrier function could have therapeutic consequences, our observations need to be substantiated by further studies to precisely delineate the mechanism of action of piroxicam (and ketoprofen) in this model system.

Colonic preparations from SAg-treated mice displayed diminished ISC responses to ETS. This treatment activates the enteric nerves and results in a transient increase in ISC because of the release of neurotransmitters that evoke Ca2+ and cAMP-dependent active Cl− secretion [18]. In addition, responses to the known cAMP-mediated Cl− secretagogues, PGE2 (receptor

![Figure 4](https://academic.oup.com/jid/article-abstract/180/5/1590/807255)

**Figure 4.** Cotreatment with ketoprofen (KET; 10⁻⁶ M) partially decreases in transepithelial resistance (TER) but does not affect diminished injected short-circuit current responses to forskolin (Isc to FSK) evoked by 24 h of exposure to conditioned medium (CM) from *Yersinia pseudotuberculosis* mitogen (YPM; 1 μg/mL)-activated peripheral blood mononuclear cells (PBMC/YPM CM; n = 3 experiments [6 monolayers]; * and **, P < .05 vs. other groups; control resistance range, 1176–1667 Ωcm²; control FSK response range, 39–74 μA/cm²).

<table>
<thead>
<tr>
<th>Change</th>
<th>Control (media only)</th>
<th>YPM-conditioned media</th>
<th>YPM-conditioned media + anti-TNF-α</th>
<th>YPM-conditioned media + anti-TNF-α and anti-IFN-γ</th>
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<tr>
<td>Resistance (Ωcm²)</td>
<td>1204 ± 47</td>
<td>827 ± 53a</td>
<td>995 ± 22b</td>
<td>1126 ± 48</td>
</tr>
<tr>
<td>Change in injected short circuit current (μA/cm²)</td>
<td>164 ± 9</td>
<td>92 ± 3a</td>
<td>99 ± 2a</td>
<td>140 ± 6</td>
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<tr>
<td>No. of epithelial monolayers</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

**Table 2.** Anti-tumor necrosis factor (TNF)-α and anti-interferon (IFN)-γ antibodies ameliorate *Yersinia pseudotuberculosis* mitogen (YPM)-induced changes in epithelial (T84) physiology.

NOTE. Data are mean ± SE. Forskolin used at final concentration of 10⁻⁵ M.

a P < .05 vs. control.

b P < .05 vs. all other groups.
mediated) and forskolin (direct adenylate cyclase effects), were reduced in tissues from YPM- and SEB-treated mice, respectively. These results complement the data from the human in vitro model and underscore the ability of YPM to affect epithelial ion transport. Moreover, these data are reminiscent of the altered murine jejunal epithelial physiology documented in response to systemic SEB administration [12] and in other models of colonic inflammation [35, 36]. For example, trinitrobenzene sulfonic acid–induced colitis reduces the ability of PGE₂ to elevate epithelial cAMP levels [37]. Colonic ion secretion occurs predominantly across enterocytes at the base of the crypts, so damage or death of these cells could account for the diminished ISC events following YPM treatment. However, colonic histology was unaffected by YPM treatment, at least in the time frame of the present study (4 h), and so distinct alterations in colonic epithelial physiology have been identified in the absence of any structural modifications. The lack of overt epithelial damage correlates with a general absence of histopathology in the jejunum of mice treated with low-dose SEB [12], and indeed SAg-induced toxic shock and subsequent mortality are minimal unless the host is primed with another agent such as lipopolysaccharide [23] or D-galactosamine [38].

Comparison of the models used revealed two notable discrepancies. First, baseline ISC was elevated in colon segments from YPM-treated mice but not in T84 monolayers exposed to YPM-activated PBMC. The mechanism underlying this discrepancy is unknown but is likely due to the presence of other cell types in vivo. For instance, nerves and stromal cells can modulate epithelial ion transport [18]. Thus, direct or indirect effects of YPM on these cell types in vivo may be responsible for the increase in baseline ISC. Second, ionic conductance across colonic segments from YPM-treated mice was not increased relative to controls, suggesting unperturbed permeability. This is contrary to observations in the in vitro model. However, an actual increase in epithelial permeability that would allow lumen-to-mucosal uptake can be masked in tissue segments examined ex vivo by opposing factors such as changes in the muscle or serosal layers. Additional in vivo permeability studies are required to examine further the ability of YPM immune activation to alter gut permeability. Despite these disparities, the juxtaposition of the in vitro and in vivo models illustrates that exposure to YPM results in immune activation and concomitant changes in colonic epithelial function.

In summary, our data demonstrate that exposure to the bacterial SAg YPM results in disruption of murine colonic ion transport in the absence of any overt colonic damage. Similar findings were observed in a human in vitro coculture model that also revealed the capacity of YPM immune stimulation to increase epithelial permeability. The disruption in T84 epithelial barrier and ion transport parameters was due to TNF-α and IFN-γ; piroxicam (and to a lesser extent ketoprofen) abrogated the increase in permeability by a mechanism that remains to be defined. The data support the postulate that bacterial SAgS are putative etiologic stimuli in enteric secretary and possibly inflammatory disorders with the potential to initiate or potentiate disease. In this context, it is noteworthy that splenocytes from YPM-challenged mice were hyperresponsive to the sensitizing SAg and also to the unrelated SAg SEB and the mitogen ConA.

Acknowledgments

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References

activated immune cells are inhibited by IL-10, but not IL-4. J Pharm Exp Ther 1998;287:128–36.


