Eur. Cytokine Netw., Vol. 14 n° 3, *September 2003*, 186–191

Th_1-Th_2 response in hyperprolactinemic mice infected with *Salmonella enterica* serovar Typhimurium

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ABSTRACT. Prolactin (PRL) is a pituitary hormone and a cytokine known to regulate several physiological functions. It plays a role in modulating the immune system of rodents and humans. A hormonal protection against listeria and salmonella infections has been previously ascribed to effects of PRL on immunocompetent cells. Here, the role of PRL in the Th1-Th2 response was evaluated based on the pattern of cytokines release by splenocytes from hyperprolactinemic mice infected with *Salmonella enterica* serovar Typhimurium.

Hyperprolactinemia by pituitary graft reduced the number of bacteria in spleens of *in vivo* infected mice. Modulation of Th1 (IFN-γ, IL-12) and Th2 (Il-4, IL-10) cytokine production by splenic cells was found. Our results indicate that PRL can up-regulate IFN-γ and IL-12 secretion in response to salmonella infection, confirming its *in vivo* immunostimulatory effect and suggesting hormonal participation in the genesis and sustenance of the Th1 response.

Keywords: *Salmonella enterica* serovar Typhimurium infection, prolactin, cytokines

INTRODUCTION

Studies indicate that PRL may interfere with immune development and function [1, 2]. Clinical, animal and *in vitro* studies combine to suggest that prolactin exhibits immunostimulatory properties [3]. However these properties ascribed to PRL have not always been consistent: some reports showed PRL to be immunusuppressive [4, 5], whereas in other instances PRL acted to enhance immune responses [6-9].

Different subtypes of immune cells from human, mice, and rats synthetize prolactin [10] and the PRL specific receptor, a member of the hematopoietin cytokine receptor superfamily, has been found on several immune cells, such as lymphocytes, macrophages, and natural killer cells, providing a mechanism for the immune actions of this hormone [1, 11]. However, results from animals with a targeted disruption of either PRL [12] or PRL-receptor gene [13] suggest that PRL is not essential for normal immune system development or function. A normal immune response to Listeria infection, involving innate as well as adaptive immune responses, also seem to be intact in the PRL-R-knockout mice, but compensatory actions by other cytokines (redundancy) in these animals have not been examined.

Certainly, PRL interacts with cytokines, and functions as a coactivator. In particular, PRL induces IL-2 receptors, promotes IL-2 release, and DNA synthesis in primary rat lymphocytes [14, 15], augments IL-2-induced proliferation of human natural killer cells [16], enhances mitogen- or LPS- stimulated production of IFN-γ by mononuclear cells [17, 18] and an interaction between high PRL concentrations and IL-12 on IFN-γ release has been observed with T cells [19]. In fact, a Th1-polarizing action of PRL has been already postulated [20], but not demonstrated, and assignment of PRL to the T helper 1 phenotype was proposed on the basis of its ability to enhance NK function, activate the interferon-regulated factor (IRF-1) transcription and interact with or generate IL-2 and IFN-γ [21].

Moreover, the suppression of PRL secretion in mice by bromocriptine increases the lethality of a Listeria challenge [22], while a treatment with PRL induces a significant, dose-dependent reduction in the mortality rate in mice infected with *S. enterica* serovar Typhimurium [23]. This latter effect may be related to a PRL-induced increase of the phagocytic capacity and intracellular killing of peritoneal macrophages [24].

In this study, we have investigated the role of PRL in the Th1-Th2 response that was evaluated by the pattern of cytokine release. A hyperprolactinemia by pituitary graft remarkably lowered the bacterial load in spleens of infected mice and a correlation between prolactin protection and modulation of Th1-(IFN-γ, IL-12) and Th2-(IL-4, IL-10) cytokine production by splenocytes is described.

MATERIALS AND METHODS

Animals: eight-week-old male BALB/C mice homozygous for the Slc11a1 gene were housed under conditions of isolation and fed sterile pellets (Mignimi 70K standard diet) and water (available *ad libitum*). All *in vivo* experiments complied with the Italian D.L. no.116 of January 27, 1992 and associated guidelines in the European Communities Council Directive of November 24, 1986 (86/609/ECC).

Bacteria: *S. enterica* serovar Typhimurium strain 74, National Collection of Type Cultures (NCTC, London NW9 5HT) was selected and grown in nutrient broth (Oxoid S.p.A. Garbagnate M.se MI) at 37°C to the logarithmic phase, suspended at a density of 10^{10} organisms per ml in phosphate-buffered saline (PBS) with 1% skimmed milk, and frozen at – 70°C in 100 µl aliquots. The viability of frozen bacteria was found to be intact when tested periodically and their virulence was equal to that of bacteria grown to the log phase.

Induction of hyperprolactinemia by pituitary grafts: fourteen days before infection, 15 mice received two pituitary glands under the kidney capsule, and 15 control mice of the same sex and age, were sham-operated by opening the kidney pocket [25, 26]. The completeness of graft acceptance was determined for each animal by autopsy at the end of the experiment.

Experimental infection: to induce the experimental infection, grafted and sham-operated (control) mice were inoculated (i.p.) with a sublethal dose (10^4 CFU/mouse) of *S. enterica* serovar Typhimurium . At various post-infection times (1-2-3-4-7 days), a group of three mice were sacrificed by cervical dislocation, and their spleens were aseptically removed.

Detection of S. enterica serovar Typhimurium in spleen cell cultures: bacterial loads in spleen were determined by plating 10-fold serial dilutions of organ homogenates on nutrient agar. Colony-forming units (CFU) were counted after overnight incubation.

Determination of PRL serum levels by biological assay: rat Nb2 lymphoma cells were cultured in RPMI 1640 medium containing 10% horse serum, 10% fetal calf serum (FCS) inactive (56°C for 30 min.), 50 mM β-mercaptoethanol, 20 mM Hepes, 500 U/ml penicillin and 500 µg/ml streptomycin. The biological assay for PRL serum determination was performed as previously described [27].

As standard, recombinant mouse prolactin, a gift from the National Institute of Diabetes and Kidney Diseases (NIDDK), was used. A standard curve for PRL concentration (from 30 pg to 10^4 pg/ml) was plotted against optical density; the hormone concentration being expressed as $\log_{10}$ pg/ml prolactin. Unknown concentrations of PRL in the serum samples were calculated by measuring the hormone concentrations off the standard curve.

The intraassay and interassay coefficients of variation were 1.3-2.9% and 3.7-6.2%, respectively.

Measurement of cytokine production by spleen cells: spleens were disrupted in RPMI 1640, supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml). Erythrocytes were lysed by 60s incubation in water. Cells were washed twice and resuspended at 2 x 10^7 cells/ml density in RPMI 1640, supplemented with 0.2 mM L-glutamine, 100 U of penicillin/ml, 100 µg/ml of streptomycin, and 20 µg of polymyxin B, and 10% heat-inactivated foetal calf serum. The viability of cells was > 98%, as determined by Trypan blue exclusion. Samples of 0.1 ml (2 × 10^6 cells/ml) were used in 96-well, flat-bottomed tissue culture plates.

The concentrations of IFN-γ, IL-12, IL-4, IL-10 in supernatants of spleen cell cultures were determined using Elisa kits (Genzyme, Cambridge, MA, USA) according to the manufacturer's recommendations. Supernatants were collected 24, 48 and 72 hours after infection and stored at – 40°C until used. The detection limits of the assays were < 1 pg/ml for IFN-γ, < 1 pg/ml for IL-4, < 13 pg/ml for IL-10, ≥ 13 pg/ml for IL-12.

Spleen cell restimulation assay

The spleen cells obtained from control and grafted animals, infected or not with *S. enterica* serovar Typhimurium were restimulated *in vitro* with Salmonella suspension at a final concentration of 10^4 cells/ml. Supernatants were collected after 24, 48, and 72 h of stimulation, and stored at – 40°C until use for cytokine determination.

Statistical analysis

All data are reported as mean ± SD values of 3 independent determinations. Statistical analysis was performed using the Anova test and multiple comparisons were made by Bonferroni's test. Statistical significance was taken as a value of $P < 0.05$.

RESULTS

Experimental infection in hyperprolactinemic mice

Control (normoprolactinemic) and grafted (hyperprolactinemic) mice were inoculated i.p. with 10^4 CFU/mouse of *S. enterica* serovar Typhimurium. The number of bacteria in the spleen was determined at various post-infection times (1-2-3-4-7 days). Bacterial counts increased in the spleens of both groups during the first 48-72 h of infection. Thereafter, grafted mice cleared the bacteria from the spleen faster than control animals (figure 1). This effect was significant at Days 4 and 7 post-infection ($P < 0.05$).

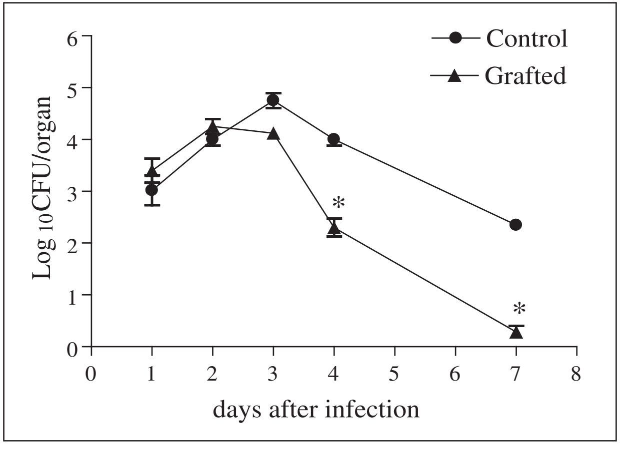

Figure 1

Viable bacteria counts in spleens of grafted or control mice at different days after infection with 10^4 CFU/mouse of *Salmonella enterica*.

Table 1
PRL serum levels (ng/ml) in grafted or control mice evaluated before and after salmonella infection. Each value is the mean ± SD of 3 animals

Groups	before infection	24	48	72
		hours after infection		
Control	4.1 ± 0.8	4.5 ± 0.5	3.3 ± 1.8	11.9 ± 2.2
Grafted	31.0 ± 5.3***	35.7 ± 2.7***	21.4 ± 0.8***	18.0 ± 6.8**

**P < 0.01 and ***P < 0.001 vs respective controls

As reported in Table 1, before and after infection, grafted mice showed significantly higher PRL serum levels than control mice ($p < 0.01$ and $p < 0.001$).

Pattern of cytokine production by spleen cells after S. enterica *serovar Typhimurium infection*

Production of different cytokines in spleen cells from sham-operated (normoprolactinemic controls) and grafted mice (hyperprolactinemic) at different times after *S. enterica* serovar Typhimurium infection are reported in figure 2.

No modification of basal levels of cytokine production by spleen cells from uninfected mice was observed throughout the course of the experiment. Moreover, these levels were not significantly modified in grafted animals.

Spleen cells from infected mice produced a measurable and time-dependent increase of IFN-γ and IL-12. In hyperprolactinemic mice, the cytokine release was significantly increased at 48 and 72 h post-infection ($p < 0.001$ for IFN-γ, and $p < 0.05$ and $p < 0.001$ for IL-12). IFN-γ release decreased thereafter and reached non-detectable levels at Day ten post-infection (data not shown).

After Salmonella infection, a very low level of IL-4 was detectable in spleen cells at 48 and 72 h post-infection. This level did not differ significantly in grafted mice. Conversely, after challenge, IL-10 production by splenocytes was enhanced in grafted mice compared to control mice at all times. This increase was significant at 48 and 72 h after infection ($p < 0.01$).

In vitro *stimulation of spleen cells from grafted and control mice, infected or not with Salmonella*

When spleen cells from non-infected mice were stimulated *in vitro* with *S. enterica* serovar Typhimurium (10^4 cells/ml), IFN-γ production was higher than that of unstimulated cells; conversely, no significant modifications were observed in IL-12, IL-4 and IL-10 production be-

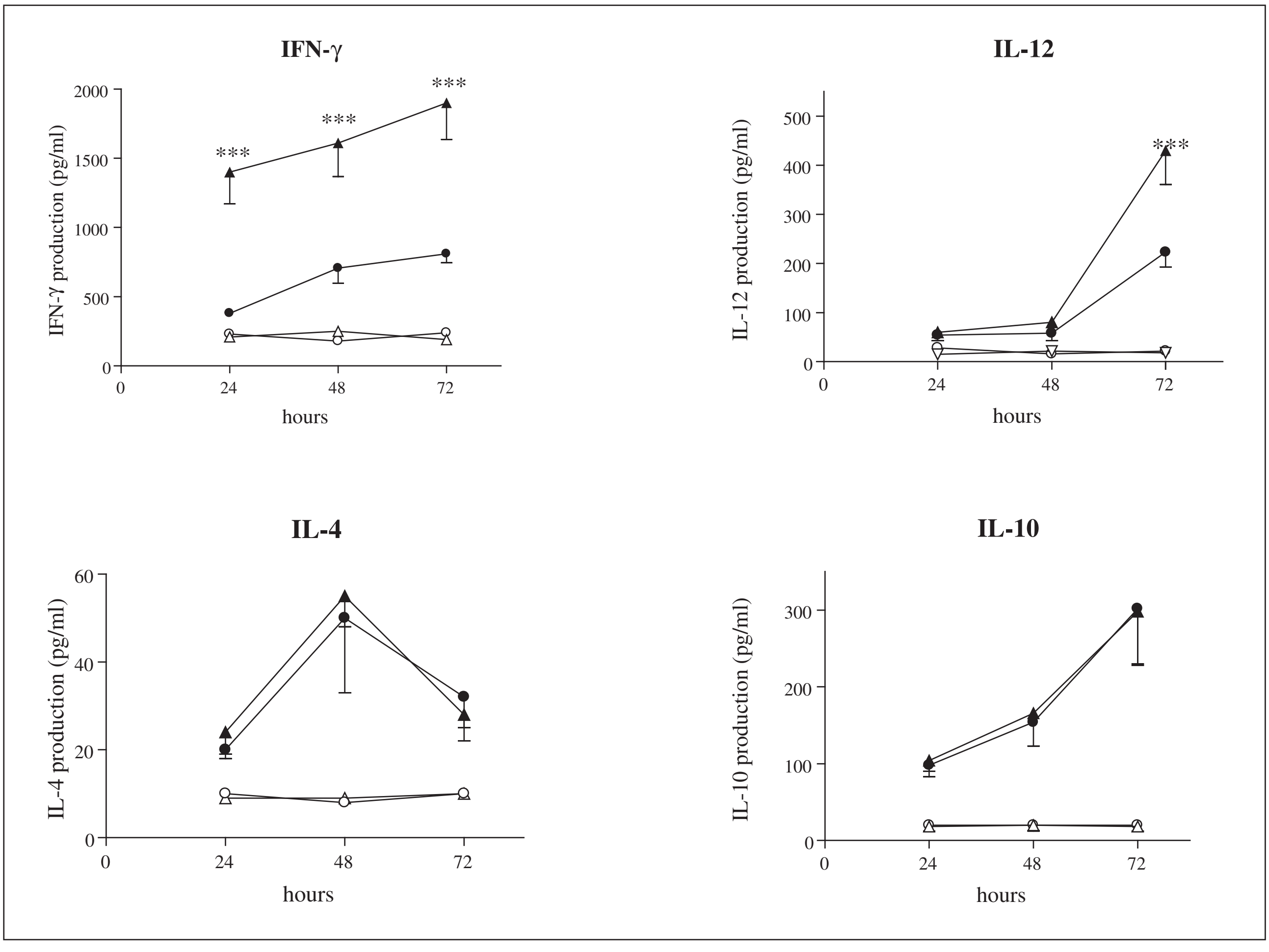

Figure 2
Production of different cytokines by spleen cells from control normoprolactinemic mice (-•-) and grafted hyperprolactinemic mice (-▲-) at different times after *Salmonella enterica* serovar Typhimurium infection. Open symbols refer to non-infected control (-○-) and grafted (-△-) mice. Data are the means ± SD of values obtained from duplicates from 3 different spleens. Cytokine production was evaluated in splenocyte supernatants after 24 hours of incubation. The levels of IL-4 release by splenocytes from non-infected control and grafted mice were not detectable.
*P < 0.05, **P < 0.01; ***P < 0.001 *versus* control.

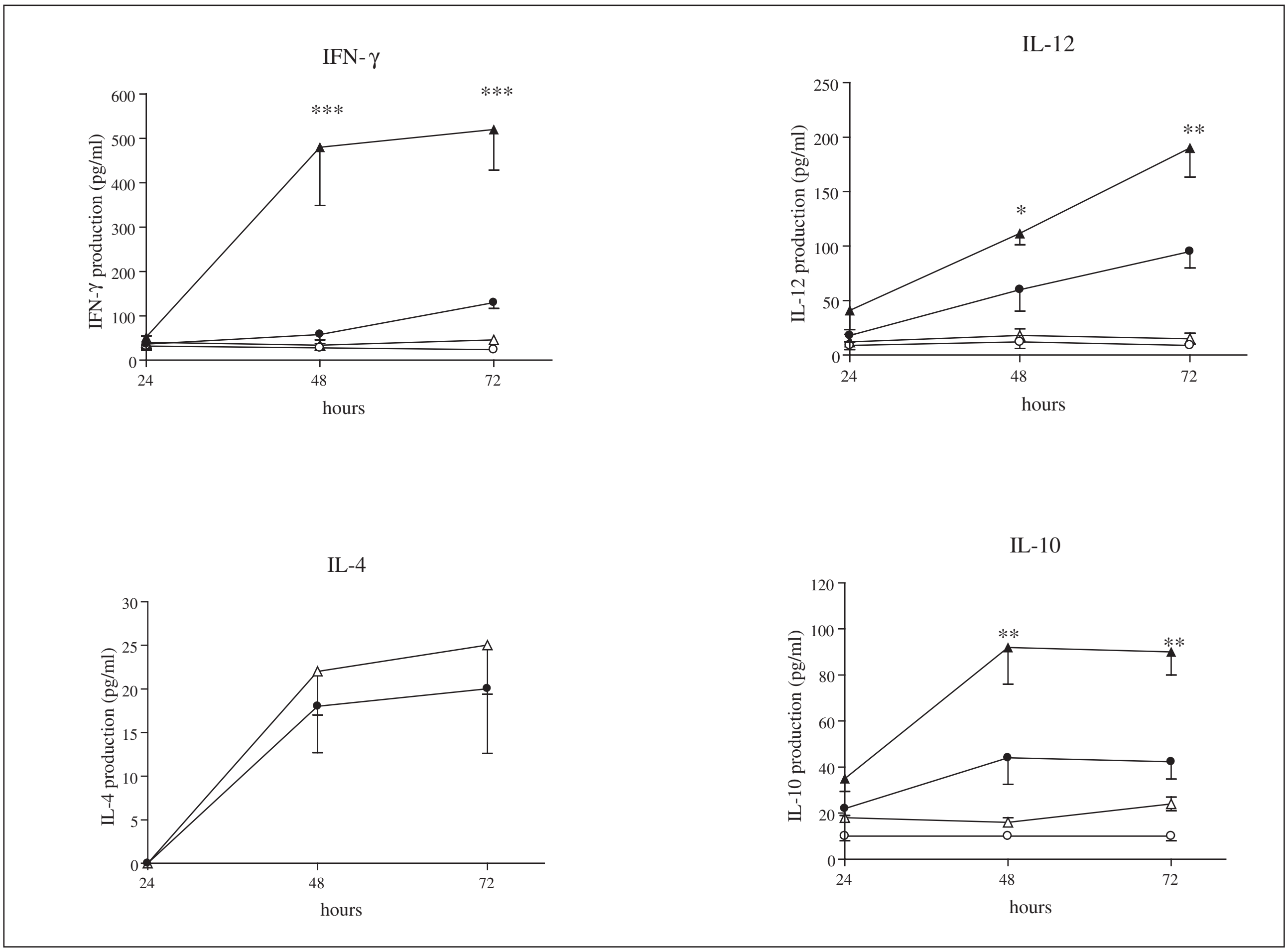

Figure 3

Cytokine production after 24h stimulation *in vitro* with *S. enterica* serovar Typhimurium (10^4 cells/ml) by spleen cells from control (-•-) or grafted (-▲-) mice, infected *in vivo* at different times with the same bacteria. Open symbols refer to values obtained from spleen cells of non-infected control (-○-) and grafted (-△-) mice and stimulated *in vitro* with salmonella. Data are means ± SD of values obtained from duplicates from 3 different spleens.
***$p < 0.001$ *versus* control.

tween unstimulated and *in vitro* stimulated cells. Hyperprolactinemia induced by grafting did not significantly modify this pattern of cytokine production (Figure 3). Spleen cells from hyperprolactinemic mice killed 24, 48, and 72 h after the *in vivo* infection and further stimulated *in vitro* with the same bacteria, produced a higher amount of IFN-γ when compared with that of spleen cells from control mice ($p < 0.001$). An enhanced IL-12 production was evident and significant when spleen cells were obtained from infected hyperprolactinemic mice killed at 72 hours after challenge ($p < 0.001$).

Low levels of IL-4 and IL-10 were detectable after *in vitro* stimulation of spleen cells prepared from the different groups of uninfected mice. After *in vitro* Salmonella challenge, a significant increase in both cytokines was observed, without any difference between normo- and hyperprolactinemic mice.

DISCUSSION

The aim of the present study was to characterise the immune responses induced by *Salmonella enterica* serovar Typhimurium in hyperprolactinemic mice in order to improve the understanding of the role of PRL in the immunological control of this infection. To exclude a possible non-hormonal activity of exogenous PRL, we induced a hyperprolactinemia by pituitary graft in mice [25, 26].

The correlation between Salmonella infection and the evoked immune response was evaluated by determining the rate of Salmonella clearance and the modifications of cytokine production by spleen cells from normo- or hyperprolactinemic, infected mice.

Our previous studies have shown that *in vivo* treatment with PRL or a hyperprolactinemia by graft, induces an increase of phagocytosis, killing and chemotaxis, as well as greater resistance to experimental infection by *S. enterica* serovar Typhimurium [23, 24]. On the other hand, many studies have shown that $CD4^+$ T cells play a major role in acquired immunity against *S. enterica* serovar Typhimurium, data confirmed using knockout mice [28-30].

In our experiments, bacterial counts increased in the spleens of both grafted and control mice during the first 72 h of infection. In grafted mice, a faster and significant reduction in the number of bacteria in the spleen was observed in comparison to control mice. A complete elimination of bacteria was evident seven days after infection. The decrease in the bacteria count may be related to the increase of several cytokines, IFN-γ, IL-12 and IL-10, in the supernatant of splenic cells. It is well established that both CD4$^+$ and CD4$^-$ cells produce IFN-γ during *S. enterica* infection, and, in particular, among the CD4$^-$ population, the NK cells are the major source of INF-γ [28, 31]. Interestingly, Lalmanach *et al.* [32] have suggested a role for IFN-γ in Salmonella infection resistance: early upregulation of IFN-γ gene expression in resistent mice lowered bacterial load. Moreover, it has very recently been demonstrated that PRL enhanced INF-γ release in peripheral whole blood after stimulation with endotoxin LPS [18].

On the other hand, IL-12 mRNA production by murine macrophages infected with Salmonella spp has been previously reported [33, 34], and this cytokine can modulate *in vivo* resistance to salmonella [29], inducing a differentiation of naive CD4$^+$ cells into the type 1 phenotype [28, 35].

PRL induces the expression of interferon regulatory factor-1 (IRF-1), which in turn increases the IL-12 p40 [1]. IRF-1 is a multifunctional immune regulator gene, that belongs to a small family of nine IRF proteins [36]. IRF-1 regulates the expression of a number of genes important for mediating antiviral and antibacterial responses [37]. It is suggested that PRL, through the JAK/Stat/IRF-1 pathway, modulates the immune response [20].

In our experimental conditions, we have shown that high levels of PRL can direct T cells toward a defined Th1 cytokine profile, increasing both the IFN-γ and IL-12 that prompt this pattern.

However, cytokine analysis also showed an increase in IL-10 production during Salmonella infection. This induction of IL-10 was not sufficient to suppress Th1 cell differentiation. Coexpression of IFN-γ and IL-10 by CD4$^+$ T cells has been reported in mice inoculated with killed *Brucella abortus* [38], and in human CD4$^+$ T cell clones primed with IL-10 [39].

In an attempt to further confirm the Th1 type polarization of the Salmonella-specific immune response in hyperprolactinemic mice, we also investigated the modifications of cytokine release by spleen cells from infected normo- or hyperprolactinemic mice upon *in vitro* stimulation with the same bacteria. A more evident amplification of the prolactin effect on Th1 cytokine production was observed. A further *in vitro* stimulation showed an amplification of the PRL effect on Th1 cytokine polarization; in fact, after this stimulation and at all time intervals examined, the effect on IL-10 production had disappeared.

These data are in agreement with previously reported PRL protection against Listeria or Salmonella infections [22-24], demonstrating a modification of immunocompetent cell function by the hyperprolactinemic state. Special interest should therefore be directed towards any hyperprolactinemia caused, for instance, by stress, infections, drugs, or some chronic disease.

ACKNOWLEDGEMENTS. We thank the NIDDK National Hormone and Pituitary Program and dr. AF Parlow for providing human prolactin, anti-human prolactin and polyclonal antibody against hGH. We are grateful to Dr. V Goffin for the generous gift of Nb2 cells.

REFERENCES

1. Yu-Lee LY. 2002. Prolactin modulation of immune and inflammatory responses. (Review) *Recent Prog. Horm. Res.* 57: 435.
2. Murphy WJ, Rui H, Longo DL. 1995. Effect of growth hormone and prolactin immune development and function. (Review) *Life Sci.* 57: 1.
3. Yu Lee LY. 1997. Molecular actions of prolactin in the immune system. (Review) *Proc Soc. Exp. Biol. Med.* 215: 35.
4. Vidaller A, Guadarrama F, Llorente L, Mendez JB, Larrea F, Villa AR, Alarcon-Segovia D. 1992. Hyperprolactinemia inhibits natural killer (NK) cell function *in vivo* and its bromocriptine treatment not only corrects it but makes it more efficient. *J. Clin. Immunol.* 12: 210.
5. Gerli R, Riccardi C, Nicoletti I, Orlandi S, Cernetti C, Spinozzi F, Rambotti P. 1987. Phenotypic and functional abnormalities of T lymphocytes in pathological hyperprolactinemia. *J. Clin. Immunol.* 7: 463.
6. Spangelo BL, Hall NR, Ross PC, Goldstein AL. 1987. Stimulation of *in vivo* antibody production and concanavalin A- induced mouse spleen cell mitogenesis by prolactin. *Immunopharmacology* 14: 11.
7. Zellweger R, Zhu XH, Wichmann MW, Ayala A, DeMaso CM, Chaundry IH. 1996. Prolactin administration following hemorragenic shock improves macrophage cytokine release capacity and decreases mortality from subsequent sepsis. *J. Immunol* 157: 5748.
8. Richards SM, Murphy WJ. 2000. Use of human prolactin as a therapeutic protein to potentiate immunohematopoietic function. (Review) *J. Neuroimmunol.* 109: 56.
9. Matera L, Mori M, Galletto A. 2001. Effect of prolactin on the antigen presenting function of monocyte-derived dendritic cells. (Review) *Lupus* 10: 728.
10. Montgomery DW. 2001. Prolactin production by immune cells. (Review) *Lupus.* 10: 665.
11. Ben-Jonathan N, Mershon JL, Allen DL, Steinmetz RW. 1996. Extrapituitary prolactin: distribution, regulation, functions and clinical aspects. (Review) *Endocr. Rev.* 17: 639.
12. Horseman ND, Zhao W, Montecino- Rodriguez E, Tanaka M, Nakashima K, Eagle SJ, Smith F, Markoff E, Dorshkind K. 1997. Defective mammopoiesis, but not hematopoieisis, in mice with a targeted disruption of the prolactin gene. *EMBO J.* 16: 6926.
13. Bouchard B, Ormandy CJ, Di Santo JP, Kelly PA. 1999. Immune system development and function in prolactin receptor-deficient mice. *J. Immunol.* 163: 576.
14. Clevenger CV, Sillman AL, Hanley-Hyde J, Prystowsky MB. 1992. Requirement for prolactin during cell cycle regulated gene expression in cloned T-lymphocytes. *Endocrinology* 130: 3216.
15. Viselli SM, Stanek EM, Mukherjee P, Hymer WC, Mastro AM. 1991. Prolactin-induced mitogenesis of lymphocytes from ovariectomized rats. *Endocrinology* 129: 983.
16. Matera L, Cesano A, Bellone G, Oberholtzer E. 1992. Modulatory effect of prolactin on the resting and mitogen-induced activity of T, B, and NK lymphocytes. *Brain Behav. Immun.* 6: 409.
17. Cesario TC, Yousefi S, Carandang G, Sadati N, Le J, Vaziri N. 1994. Enhanced yields of gamma interferon in prolactin treated human peripheral blood mononuclear cells. *Proc. Soc. Exp. Biol. Med.* 205: 89.
18. Breidthardt T, Frohn C, Luhm J, Kirchner H, Brand JM. 2002. Prolactin induces enhanced interferon gamma release in peripheral whole blood after stimulation with either PHA or LPS. *Immunoobiology.* 206: 424.
19. Matera L, Mori M. 2000. Cooperation of pituitary hormone prolactin with interleukin-2 and interleukin-12 on production of interferon-gamma by natural killer and T cells. *Ann. N.Y. Acad. Sci.* 917: 505.

20. Yu Lee L, Luo G, Moutoussamy S, Finidori J. 1998. Prolactin and growth hormone signal transduction in lymphoemopoietic cells. (Review) *Cell Mol Life Sci.* 54: 1067.
21. Matera L. 1997 Action of pituitary and lymphocyte prolactin. (Review) *Neuroimmunomodulation.* 4: 171.
22. Bernton EW, Meltzer MS, Holaday JW. 1988. Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. *Science* 239: 401.
23. Di Carlo R, Meli R, Galdiero M, Nuzzo I, Bentivoglio C, Carratelli CR. 1993. Prolactin protection against lethal effects of *Salmonella typhimurium. Life Sci.* 53: 981.
24. Meli R, Raso GM, Bentivoglio C, Nuzzo I, Galdiero M, Di Carlo R. 1996. Recombinant human prolactin induces protection against *Salmonella typhimurium* infection in the mouse: role of nitric oxide. *Immunopharmacology* 34, 1.
25. Adler RA. 1986. The antherior pituitary- grafted rat: a valid model of chronic hyperprolactinemia. (Review) *Endocr. Rev.* 7: 302.
26. Cross RJ, Campbell JL, Roszman TL. 1989. Potentiation of antiboby responsiveness after the transplantation of a syngeneic pituitary gland. *J. Neuroimmunol.* 25: 29.
27. Pacilio M, Migliaresi S, Meli R, Ambrosone L, Bigliardo B, Di Carlo R. 2001. Elevated bioactive prolactin levels in systemic lupus erythematosus-association with disease activity. *J. Rheumatol.* 28: 2216.
28. Pie S, Truffa-Bachi P, Pla M, Nauciel C. 1997. Th1 response in *Salmonella typhimurium*-infected mice with a high or low rate of bacterial clearance. *Infect. Immun.* 65: 4509.
29. Galdiero M, De Martino L, Marcatili A, Nuzzo I, Vitiello M, Cipollaro de l'Ero G. 1998. Th1 and Th2 cell involvement in immune response to *Salmonella typhimurium* porins. *Immunology* 94: 5.
30. Gupta S. 1998. Priming of T-cell responses in mice by porins of *Salmonella typhimurium. Scand. J. Immunol.* 48: 136.
31. Bancroft GJ. 1993. The role of natural killer cells in innate resistance to infection. (Review) *Curr. Opin. Immunol.* 5: 503.
32. Lalmanach AC, Montagne A, Menanteau P, Lantier F. 2001. Effect of the mouse Nramp1 genotype on the expression of IFN-gamma gene in early response to Salmonella infection. *Microbes Infect.* 3: 639.
33. Chong C, Bost KL, Clements JD. 1996. Differential production of interleukin-12 mRNA by murine macrophages in response to viable or killed Salmonella spp. *Infect. Immun.* 64: 1154.
34. John B, Rajagopal D, Pashine A, Rath S, George A, Bal V. 2002. Role of IL-12-independent and IL-12-dependent pathways in regulating generation of the IFN-gamma component of T cell responses to *Salmonella typhimurium*. *J. Immunol.* 169: 2545.
35. Mosmann TR, Sad S. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. (Review) *Immunol. Today* 17: 138.
36. Sato M, Taniguchi T, Tanaka N. 2001. The interferon system and interferon regulatory factor transcription factors – studies from gene knockout mice. (Review) *Cytokine Growth Factor Rev* 12:133.
37. Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. 2001. IRF family of transcription factors as regulators of host defense. (Review) *Annu Rev Immunol* 19: 623.
38. Svetic A, Jian YC, Lu P, Finkelman FD, Gause WC. 1993. *Brucella abortus* induces a novel cytokine gene expression pattern characterized by elevated IL-10 and IFN-gamma in CD4 + T cells. *Int. Immunol.* 5: 877.
39. Gerosa F, Paganin C, Peritt D, Paiola F, Scupoli MT, Aste-Amezaga M, Frank I, Trinchieri G. 1996. Interleukin-12 primes human CD4 and CD8 T cell clones for high production of both interferon-gamma and interleukin-10. *J. Exp. Med.* 183: 2559.