# Evidence for a role of IFN γ in control of *Listeria monocytogenes* in T cell deficient mice

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*Summary.* The role of interferon (IFN) γ in controlling chronic infections of *Listeria monocytogenes* (Listeria) was studied in athymic C57BL/6 *nu/nu* mice, and by treating thymectomized C57BL/6 +/+ mice with monoclonal rat CD4 and CD8-specific monoclonal antibodies (Mab). Mice treated with a combination of the two T cell subset antibodies were similar to athymic, nude mice in being able to contol Listeria infection, keeping the titers below 3–5 $\log_{10}$ bacteria per organ, but they could not eliminate them completely. Treatment with antibodies to IFN γ of nude or $CD4^+ + CD8^+$ – T cell-depleted mice suffering from chronic Listeria infection caused a marked increase of Listeria titers in liver and spleen. This result implies a role of IFN γ in maintaining anti-Listeria resistance in mice lacking mature T cells.
*Key words.* T cell deficient mice; nude mice; T subset depleted mice; interferon γ; *Listeria monocytogenes.*

Antibacterial cell-mediated immunity is biphasic; first, resident macrophages limit early bacterial growth, and then, subsequently to the induction of a specific T cell response, mononuclear phagocytes are attracted by T cell products (lymphokines) to the sites of bacterial proliferation and are stimulated by them to express increased bactericidal activity[1–3]. The function of T cells and lymphokines (e.g. interferon IFN γ) in the activation of antibacterial host defense during Listeria infections has been well documented[2,4–7]. The role of lymphokines in controlling Listeria in T cell-deficient mice is less clear[8–10]. Several studies have shown that nude mice are able to control bacterial titers at a certain level over an extended period of time despite the absence of significant populations of mature T cells[10–13]. This study attempted to evaluate the role of IFN γ in the apparent long-term control of Listeria in mice lacking mature T cells.

## *Materials and methods*

*Mice.* Six- to eight-week-old inbred C57BL/6 ($H-2^b$) +/+ and *nu/nu* mice were bought from Bomholtgard, Ry, Denmark. The +/+ mice exhibited high-intermediate resistance to Listeria as described by Cheers[14].

*Antibodies, thymectomy and T cell depletion.* The rat IgG 2b, monoclonal antibodies YTS 169.4 (CD8-specific Mab) and YTS 191.1 (CD4-specific Mab) were prepared as described[15,16]. Mice were treated with about 1 mg of active antibody as indicated in the experiments. Depletion was > 97% for both the CD4 and CD8 T cell populations. The sheep anti-murine IFN γ antiserum and treatment of mice has been described in detail elsewhere[17]. Mice were thymectomized at the age of 6–8 weeks according to the method of Monaco et al.[18]. The animals were allowed to recover for at least 4 weeks before they were treated twice with antibody.

*Culturing and enumeration of bacteria.* A seed-culture of *Listeria monocytogenes* was originally obtained from Dr R. V. Blanden, Australian National University, Canberra[4]; the lethal dose 50% was about $1.5 \times 10^5$ cfu for C57BL/6 +/+ mice. Methods of culture and enumeration were as described[3,4,17].

*Production and assay of IFN γ.* Unfractionated spleen cells from untreated thymectomized C57BL/6 mice or from those which were injected with CD4-specific Mab, with CD8 Mab or with a combination of the two anti-T cell subset antibodies were adjusted to a concentration of $5 \times 10^6$ lymphocytes/ml in Iscove's modification of Dulbecco's medium (IMDM) supplemented with 5% FCS, $10^{-5}$ M 2-mercaptoethanol, 100 U/ml of penicillin, streptomycin and 2 mM L-glutamine. Spleen cell suspensions were transferred to a 24-well plate and stimulated for 48 h with 5 µg/ml of concanavalin A (Pharmacia, Uppsala, Sweden). IFN γ was measured by a solid phase sandwich immunoassay method[16,19] using the rat anti-IFN γ Mabs R4-6A2 and An-18 (kindly provided by Dr E. Havell, Trudeau Institute, Saranac Lake, NY, and Dr S. Landolfo, University of Turin, Turin, Italy).

## *Results and discussion*

*Comparison of resistance to Listeria in $CD4^+ + CD8^+$ T cell-depleted mice and athymic mice.* Thymectomized C57BL/6 mice were treated with a combination of CD8 and CD4-specific Mab 14 and 10 days prior to infection with 400 cfu of Listeria. Mice were sacrificed 6 and 12 days after bacterial challenge and Listeria titers were measured in the liver and spleen. At both time points the values assessed in $CD4^+ + CD8^+$ cell-depleted mice were comparable to those found in athymic, T cell-incompetent mice. Mice treated with both the anti-T cell subset antibodies, and also nude mice, were able to control the spread of Listeria to a certain level but were not able to completely eliminate the bacterium (table 1).
No significant differences could be observed between thymectomized but otherwise normal mice and thymec-

Table 1. Antibacterial host defense in killer plus helper T cell-depleted C57BL/6 mice and influence of anti-IFN $\gamma$ antibodies on host-Listeria balance in the absence of mature $CD8^+$ and $CD4^+$ T cells.

| Mouse strain Treatment | Listeria titer ($log_{10}$) per organ ± SEM Day 6 | | Day 12 | | Day 15 Anti-IFN $\gamma$ | | Normal serum | |
|---|---|---|---|---|---|---|---|---|
| | Liver | Spleen | Liver | Spleen | Liver | Spleen | Liver | Spleen |
| C57BL/6 +/+ Thymectomized anti-CD4/CD8 | 3.1 + 0.3 | 4.2 + 0.3 | 3.5 + 0.4 | 4.4 + 0.3 | 5.8 + 0.6 | 6.6 + 0.7 | 3.6 + 0.4 | 4.7 + 0.3 |
| C57BL/6 *nu/nu* none | 3.3 + 0.2 | 4.3 + 0.3 | 3.4 + 0.3 | 4.7 + 0.4 | 6.2 + 0.5 | 7.1 + 0.6 | 3.7 + 0.5 | 4.8 + 0.4 |
| C57BL/6 +/+ Thymectomized none | 2.4 + 0.3 | 3.2 + 0.2 | < 1.5 | < 1.5 | < 1.5 | < 1.5 | < 1.5 | < 1.5 |

Thymectomized C57BL/6 mice were injected with CD4 and CD8-specific Mab antibodies 10 and 14 days prior to infection with 400 cfu of Listeria. Groups of mice were killed 6 and 12 days later. The remaining infected, $CD4^+/CD8^+$ cell-depleted mice and nude mice were treated with $2 \times 10^4$ neutralizing units of anti-IFN $\gamma$ on day 13 and 14 after bacterial challenge; Listeria titers were assayed 24 h later. Indicated values represent the mean of five individual organ samples ± SEM. The results are representative for two similar experiments.

tomized animals treated with a combination of the two anti-T cell subset antibodies when measuring the bacterial titer in the blood 5 min and in the spleen and liver 60 min after i. v. infection with $6 \times 10^4$ cfu of Listeria (not shown). To assess whether IFN $\gamma$ played a role in the anti-Listeria activity expressed in $CD4^+ + CD8^+$ T cell-depleted mice or in nude mice, animals of the same experimental groups were treated with a polyclonal anti-IFN $\gamma$ antiserum or normal serum on days 13 and 14 after Listeria infection, and were killed 24 h later (table 1; day-15 groups). $CD4^+ + CD8^+$ T cell-depleted, thymectomized C57BL/6 mice and athymic C57BL/6 *nu/nu* mice treated in addition with anti-IFN $\gamma$ had Listeria titers that were 2 log units higher than those in identically pretreated control mice injected with normal serum.

*Effect of T cell subset depletion on capacity to produce IFN $\gamma$.* Spleen cell populations ($5 \times 10^6$ cells) of T cell subset-depleted mice were stimulated in vitro with 5 µg/ml concanavalin A and the production of IFN $\gamma$ in the culture supernatants was assessed 48 h later. Spleen cells from thymectomized and normal C57BL/6 mice treated with CD4-specific Mab produced approximately normal amounts of IFN $\gamma$ (table 2). Mice treated with CD8-specific Mab or a combination of the two Mab secreted reduced amounts of IFN $\gamma$ (table 2) but its production was not completely abolished.

When treated with specific anti-IFN $\gamma$ antiserum athymic nude mice and animals depleted of both $CD4^+$ and $CD8^+$ T cell subsets were no longer able to control low dose infections of Listeria at the usual relatively stable levels of infection[10]. Treatment with sheep anti-IFN $\gamma$ antiserum 13 and 14 days after initiation of infection caused an about 100-fold increase of Listeria titers in spleens and livers of T cell-deficient mice within 24–48 h. The results obtained with this treatment suggest a drastically impaired production of IFN $\gamma$ in vivo in the absence of mature T cells in nude mice, or after T cells had been drastically reduced in TX mice; in contrast, in vitro cultured spleen cells from $CD4^+ + CD8^+$-depleted TX mice still produced some IFN $\gamma$ (table 2). These differences are not easily explained but probably reflect differing readouts and differing sensitivities of the in vivo vs in vitro test systems.

Table 2. Capacity of T cell subset-depleted spleen cell populations to secrete IFN $\gamma$ after stimulation with concanavalin A

| Mab treatment of mice | IFN $\gamma$ release into the supernatant (U/ml) | |
|---|---|---|
| | Cells of normal mice | Cells of thymectomized mice |
| CD8-specific Mab | 102 ± 13 | 75 ± 21 |
| CD4-specific Mab | 194 ± 25 | 168 ± 16 |
| CD4 + CD8-specific Mab | 35 ± 8 | 16 ± 9 |
| None | 182 ± 18 | 137 ± 16 |

Spleen cells from normal or thymectomized C57BL/6 mice were stimulated in vitro with concanavalin A for 48 h. Subsequently the IFN $\gamma$ released into the supernatant was titrated in a two-site 'sandwich immunoassay'. Results are representative for two similar experiments. Values represent means ± SEM of three cultures.

Taken together, the findings presented indicate that IFN $\gamma$ is involved in the partial, and to a certain extent efficient, control of Listeria replication in both the liver and spleen of T cell-deficient mice. This result supports earlier studies by Nakane et al.[24] demonstrating that anti-IFN $\gamma$, given on day 0 and day 1 of infection, significantly suppressed the Listeria resistance of normal immunocompetent mice. The present results apparently contradict experiments[10] showing that treatment of nude mice suffering from chronic listeriosis with recombinant IFN $\gamma$ dit not detectably decrease Listeria titers. The explanation may be that injection of additional IFN $\gamma$ cannot enhance anti-Listeria activity beyond that normally seen in nude mice. However, recombinant IFN $\gamma$ has been shown to enhance the resistance of neonatal mice[9] against Listeria, although it is not clear whether the effect is related to T cell-deficiency or to the immature status of phagocytes in these mice.

Although rather indirectly, these findings may give some indication of the role of class I restricted T cells in immunity to Listeria in normal mice[20–23]. Since these effector cells may produce IFN $\gamma$[24] as do some T helper cells[25] their protective role may be mediated by IFN $\gamma$ and not necessarily via cytotoxicity[20–23], particularly if some of this T cell activity is not always classically transplantation antigen-restricted[22].

Collectively, the evidence discussed, and the experiments presented here, using a low dose of infection and a potent neutralizing anti-IFN $\gamma$ antiserum, indicated that IFN $\gamma$

may play a crucial role in controlling Listeria in T cell incompetent mice.

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## Brominated diphenyl ethers from a marine bacterium associated with the sponge *Dysidea* sp.

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*Summary.* Bacteria *Vibrio* sp. isolated from the sponge *Dysidea* sp. were shown to biosynthesize brominated diphenyl ethers. We identified one of the bacterial brominated metabolites, using gas liquid chromatography and mass spectrometry to compare this product with standard 3,5-dibromo-2-(3',5'-dibromo-2'-methoxyphenoxy)phenol. The latter has been isolated from ethanol extracts of the sponge *Dysidea* sp.
*Key words.* Marine bacteria; sponges; *Dysidea sp.*

In recent years, several reports have appeared in the literature about the microbial biosynthesis of some metabolites which had earlier been isolated from marine macroorganisms[1-3]. In the present work we show that symbiotic microorganisms of the sponge *Dysidea* sp. can synthesize physiologically active compounds which belong to the group of brominated diphenyl ethers. To identify brominated bacterial metabolites we employed tetrabrominated diphenyl ether **1**, isolated from the ethanol extract of the sponge *Dysidea* sp. as a standard. The $H^1$ and $C^{13}$ NMR spectra of **1** were identical to those of 3,5-dibromo-2-(3',5'-dibromo-2'-methoxyphenoxy)phenol from *Dysidea fragilis*[4].

Eight pure bacterial cultures were isolated from two specimens of *Dysidea* sp. collected near the islands Tutuila and Ofu (Eastern Samoa) in June 1989, during the 9th cruise of the R/V "Akademik Oparin". All the bacteria isolated were grown during 96–100 h at 30 °C in a thermostat in 1-l flasks with 500 ml of medium (peptone 5 g, yeast extracts 2.5 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, seawater 1 l). Evaporated butanol extracts from culture broths were tested for the presence of brominated metabolites, using negative ion mass spectrometry (NIMS) with direct inlet for detection of characteristic ions of Br at m/z 79 and 81. Thus the presence of brominated compounds was demonstrated in the culture broths of two bacterial strains of the genus *Vibrio*. One of these strains, KMM 9-81-1, was cultivated under the above conditions.

The filtered culture broth was extracted by n-butanol and the butanolic extract evaporated in vacuo. The dry concentrate was fractionated in the system: water:ethanol:chloroform (1:1:1). Substances that gave a color reaction with diazotized benzidine, characteristic for phenol compounds, were present in the chloroform