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Milk from hyperimmunized dairy cows as a source of a novel biological response modifier

D. J. Ormrod and T. E. Miller

Department of Medicine, University Of Auckland, Auckland, New Zealand

Abstract

Laboratory investigations have established that hyperimmunization of dairy cows with a polyvalent bacterial vaccine results in the secretion of biologically active substances into the milk. One of these factors, a low molecular weight anti-inflammatory agent (HIMF), has been studied in detail. The evidence supports the hypothesis that HIMF suppresses inflammation by inhibiting neutrophil emigration. Additionally, the experiments suggested that HIMF was capable of modifying the host response to infection and lymphocyte function. These effects have considerable clinical potential and were therefore investigated further. Intravenous administration of HIMF to rats with subcutaneous *E. coli* infection reduced the influx of neutrophils in the early phase of infection by as much as 73%. HIMF suppressed the host vs. graft but not the graft vs. host reaction and resulted in an increase in spleen weight and the number of splenic lymphocytes. The lymphocyte response to concanavalin A was also abrogated by the agent. These data indicate that HIMF may be useful for the inhibition of tissue destructive infectious processes, and in situations where suppression of lymphocyte function is desirable.

Introduction

Mammalian milk is of considerable benefit to the offspring. The protective effect extends beyond that of providing nutrition and passive immunity to infection, and a role for anti-inflammatory factors in milk in promoting survival is now recognized [1–3]. Studies have shown that hyperimmunizing dairy cows with a killed bacterial vaccine results in the secretion of pharmacologically active substances into the milk, including a potent anti-inflammatory moiety [4]. This hyperimmune milk anti-inflammatory factor (HIMF) was shown to suppress inflammation by inhibiting neutrophil emigration from the vasculature [5, 6]. The present paper describes additional effects of HIMF which have been identified during the course of these studies. Specifically, they address the suppression of tissue destructive inflammatory processes in infection and the inhibition of lymphocyte function.

Address for correspondence: Douglas Ormrod, Department of Medicine, Fourth floor, Auckland Hospital, Auckland, New Zealand.

Methods, results and discussion

Suppression of infection-associated inflammation by HIMF

Ninety Dark Agouti (DA) rats were divided into two groups of 45. One group was untreated and served as controls, while individuals in the second group were given 40 mg of HIMF i.v. Sponges were

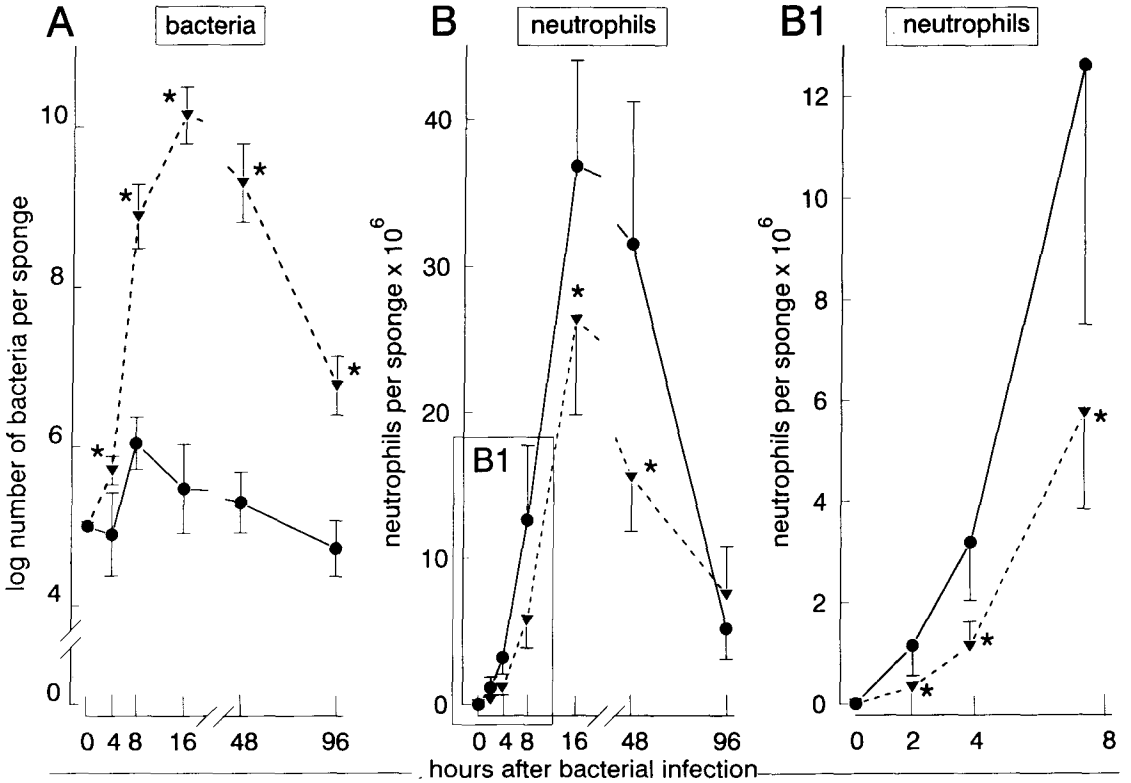

Figure 1
HIMF, given i.v. at 40 mg per animal, results in enhanced bacterial replication in subcutaneously implanted, *E. coli*-infected sponges (A). The increase in bacterial numbers is paralleled by reduced emigration of neutrophils into the sponge (B) which is particularly marked during the early phase of infection (B1). $n = 6$–8, $^*p < 0.01$.

implanted subcutaneously in all animals and, at the time of implantation, each sponge was inoculated with 100 000 viable *E. coli* 075. Groups of 6–8 animals were killed at intervals thereafter and the sponges examined bacteriologically and for fluid and cellular content. The rate of bacterial replication was up to 10 000 times greater in HIMF-treated animals than in the controls (Fig. 1A). The marked inhibition of the cellular infiltrate into the infected sponges in HIMF-treated animals provides a likely explanation for these findings (Fig. 1B). The early response to infection is a critical factor determining the outcome of an infectious episode and also influences the degree of tissue damage. In this experiment, 2, 4 and 8 h after challenge, the cellular infiltrate in those animals given HIMF was 27%, 35% and 46%, respectively, of the infiltrate in control animals (Fig. 1B).

Previous studies, with cyclosporin A and methylprednisolone, showed a similar association between suppression of the acute cellular infiltrate and the promotion of bacterial infection [7, 8]. However, the increased bacterial load promoted a rebound response and between 24 and 80 h post challenge a massive influx of neutrophils occurred. When tissue was involved, the enhanced inflammatory response resulted in a marked exacerbation of tissue damage and scarring [8]. Although HIMF suppressed the early inflammatory response and was associated with a 10 000 fold increase in bacterial numbers, no rebound effect was found. Agents with the ability to suppress inflammation in infection without enhancing tissue damage obviously have considerable potential and further experiments, using HIMF in clinically relevant models of infection, are planned.

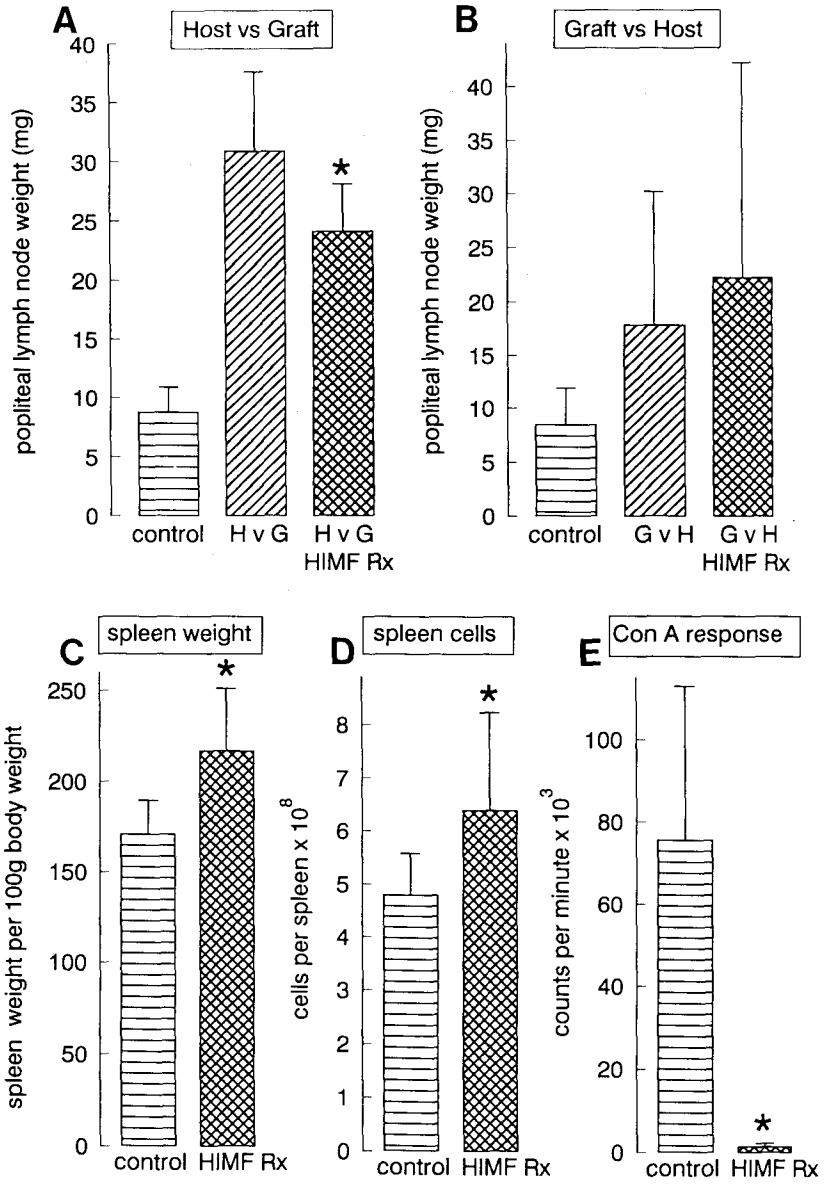

Figure 2
Modification of lymphocyte function by i.v. injected HIMF. For A, B, C, D and E, respectively, $n = 12$, 50, 32, 30 and 6. $*p < 0.01$.

Effect of HIMF on lymphocyte function

The ability of HIMF to induce a reversible decrease in the number of circulating lymphocytes [6] prompted the further investigation of the effect of the agent on lymphocyte function. The Host versus Graft (HvG) and Graft versus Host (GvH) assays were used to determine the effect on T lymphocyte function. In the HvG analysis, parental DA animals were injected i.v. with 20 mg of HIMF 48, 24 and 3 h before lymphocytes from their F1 hybrid offspring (DA × Hooded Oxford) were injected into

their footpads. The response was quantified by removing and weighing the popliteal lymph nodes. Thus, the effect of HIMF on the ability of T lymphocytes from the intact host (DA) to respond to the foreign histocompatibility antigens of the F1 lymphocytes was measured. The protocol produced a highly significant reduction (30%) in the response (Fig. 2A). In the GvH reaction lymphocytes were obtained from HIMF-treated parental rats (DA) and injected into the footpads of their F1 DA × HO offspring. This assay measured the *in vivo* responsiveness of T lymphocytes removed from the host under evaluation (i.e. HIMF-treated). The HIMF regimen had no effect on the GvH response (Fig. 2B).

During the preceding experiments an apparent increase in the number of splenic lymphocytes in HIMF-treated animals was noted. Further experiments showed a significant increase in both spleen weight and in spleen cell numbers (Figs. 2C and 2D). Interestingly, the increase in spleen cell numbers was approximately equal to the decrease in the number of circulating cells reported previously [6]. Finally, the effect of HIMF on the ability of isolated splenic lymphocytes to respond to the mitogen concanavalin A was determined. HIMF, administered as for the GvH and HvG experiments, almost totally abrogated the mitogenic response of cultured lymphocytes to concanavalin A (Fig. 2E).

These results provide strong evidence for an effect of HIMF on lymphocyte function, which is currently being pursued.

Acknowledgement

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