Effects of tilomisole, indomethacin and levamisole on regulation of Epstein Barr virus-induced B cell proliferation by peripheral blood mononuclear cells from normal individuals and patients with rheumatoid arthritis

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Abstract
When activated in autologous mixed leukocyte reactions (auto-MLR) in vitro, T cells from normal individuals produce a suppressor factor(s) which inhibits the Epstein-Barr virus (EBV)-induced proliferation of normal B cells. In contrast, T cells from patients with rheumatoid arthritis (RA) are deficient in their ability to generate this suppressor factor in auto-MLR. Addition of tilomisole (Wy-18,251; 3-(p-chlorophenyl)thiazolo[3,2-a]benzimidazole-2-acetic acid) to the auto-MLR (0.1–100 μg/ml) did not alter the production of suppressor activity by normal T cells, but 100 μg/ml tilomisole restored to normal the defective factor production by RA T cells. Indomethacin (1 μg/ml) but not levamisole (0.1–100 μg/ml) had a similar effect, which suggests that the action of tilomisole in this system is due to its ability to inhibit prostaglandin biosynthesis. Nonetheless, the ability of tilomisole to down-regulate B cell function may contribute to the compound's antiarthritic activity.

Introduction
Several immune abnormalities have been reported in patients with rheumatoid arthritis (RA) and it has been proposed that pharmacological agents which improve or restore to normal these immunological defects could be therapeutically beneficial [1]. Recent studies have indicated that T cells from RA patients are defective in their ability to regulate Epstein-Barr virus (EBV)-induced B cell proliferation [2]. Normal human B cells exposed to EBV in vitro undergo blast transformation and cell proliferation. The amount of B cell proliferation which occurs is modulated by T lymphocytes; normal T cells inhibit EBV-induced proliferation of B cells, but T cells from patients with RA do not regulate in vitro B cell proliferation as efficiently as do normal T cells [2]. Furthermore, the suppression of B cell proliferation is due to the production of a suppressor factor (SF) by T cells. Thus, normal T cells produce high levels of suppressor factor(s) whereas RA T cells produce diminished amounts and are, therefore, less able to inhibit B cell proliferation [3].

Tilomisole (Wy-18,251) is an immunomodulatory agent which is currently in phase I/II clinical trials [4–7]. In this study, we assessed the ability of tilomisole to modulate the defective regulation of B cell activity by RA T cells in vitro.

Materials and methods
Autologous mixed leukocyte reactions (auto-MLR) were prepared using T cells and mitomycin C treated non-T cells from the peripheral blood of RA patients and age- and sex-matched normal
volunteers as described previously [2]. After 72 hours of incubation at 37°C, the supernatant fluids from these cultures were collected, filtered, and assayed for suppressor activity by their ability to inhibit EBV-induced proliferation ([3H]thymidine uptake) on day 10 of culture as described in detail elsewhere [2, 3].

Where indicated, tilomisole (synthesized by Dr. P. Wei, Wyeth Laboratories, Inc., Radnor, PA), levamisole (Aldrich Chemical Co., Milwaukee, WI) or indomethacin (Merck, Rahway, NJ) were added (0.1–100 μg/ml) to the auto-MLR at the start of the 72 hour culture period. None of these drugs had any direct effect on the EBV-induced B cell proliferation assay itself.

**Results and discussion**

Figure 1 shows that tilomisole did not significantly affect the production of suppressor activity by normal T cells in auto-MLR cultures. However, 100 μg/ml of tilomisole restored to normal levels the production of suppressor activity in auto-MLR cultures of RA T cells (Figure 1). Tilomisole (100 μg/ml) did not directly affect the proliferation when added directly to EBV-infected B cells (data not shown). Indomethacin (1 μg/ml) also restored to normal the reduced suppressor factor production in RA auto-MLR, and, like tilomisole, indomethacin had no significant effects on normal auto-MLR [8]. In contrast, levamisole (0.1–100 μg/ml) failed to significantly alter suppressor factor production by either normal or RA T cells in auto-MLR cultures (Figure 2).

To date, nearly all studies of tilomisole have involved normal lymphocytes [7, 8]. However, this study indicates that tilomisole can, indeed, have positive immunoregulatory effects on lymphocytes from patients with autoimmune disease (i.e., rheumatoid arthritis). The effects of tilomisole in this model system were very similar to