

Suppression of Natural Xenophile Antibodies With the Novel Immunomodulating Drug Leflunomide

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NATURAL xenophile antibodies (NXA) and induced xenophile antibodies (XA) are considered a major barrier to organ transplantation between phylogenetically discordant species and are held to be responsible for hyperacute and acute rejection of xenogeneic organs.¹ The aims of this study were (1) to analyze the kinetics of NXA and XA in the xenogeneic model "rat antihuman," which allows experimental manipulation, and (2) to manipulate NXA and XA production by the new immunomodulating drug leflunomide (LF). LF is thought to preferentially inhibit T-dependent B-lymphocyte proliferation by antagonizing a T-helper cell-derived soluble, B-cell activating factor, possibly an interleukin.²

MATERIALS AND METHODS

Animals and Xenogeneic Sensitization

Two- to three-month-old male LEW rats received 2×10^7 human peripheral blood lymphocytes (PBL) in 2 mL RPMI-1640 (IP) on day 0.

LF Treatment

A77 1726, the water-soluble metabolite of LF (Hoechst Company, Kalle-Albert, Wiesbaden, Germany), was applied daily IP from day 0 to day +10 at either 3 or 10 mg/kg/d. LF was tolerated well by all individuals, even in the relatively high dose of 10 mg/kg.

Sampling of Test Sera

LEW sera were prepared from tail vein blood at regular intervals posttreatment, stored at -80°C , and heat inactivated before use to eliminate complement function.

Analysis of NXA and XA

Rat sera were titrated and added to vital human PBL for 45 min/ 20°C . After extensive washings, fluorescein isothiocyanate (FITC)-conjugated goat antirat IgG or IgM were added to PBL as secondary antibodies. NXA and XA binding was quantitated by flow cytometry using a FACScan (Becton Dickinson).

RESULTS

NXA in Nonsensitized Rats

Male LEW rats have NXA in their sera that bind to viable human PBL, mostly with a median IgM titer of 1:4 and a median IgG titer of $<1:1$. This observation refers to 31 of 40 normal rat sera, where binding of IgM was markedly stronger than binding of IgG, as expected. In 7 of 40 sera, however, binding of IgM was equal for binding of IgG and in 2 of 40 sera it was weaker.

LF Treatment of Nonsensitized Rats

Nonsensitized rats that receive 10 mg/kg/d LF for 10 days ($n = 8$) show a decrease of NXA binding on day +11

(about 30% reduction of IgG; about 50% reduction of IgM). NXA titers rise again when LF treatment is terminated, however: (1) above normal (presumably as a rebound effect) up to day +40 and (2) back to normal beyond day +40.

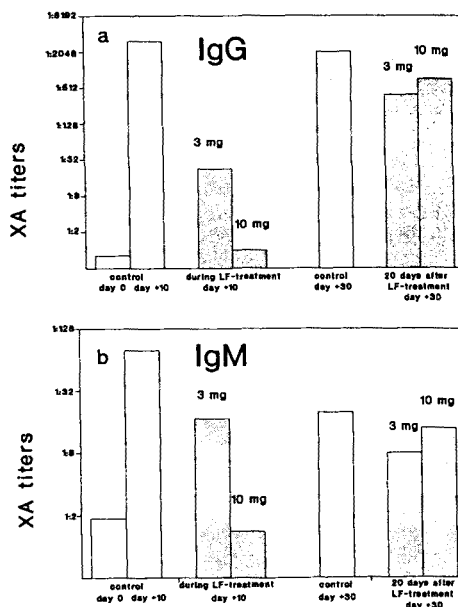


Fig 1. Dose-dependent inhibition of xenogeneic sensitization (rat antihuman PBL model) by LF. Male LEW rats were sensitized on day 0 with 2×10^7 human PBL IP and received either 3 mg/kg or 10 mg/kg LF IP from day 0 to day +10. IgG (a) and IgM (b) titers of XA in sera of sensitized and LF-treated rats were measured by flow cytometry either immediately on day +10, or 20 days after the end of LF treatment on day +30; $n = 5$ per experiment.

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XA in Sensitized Rats

A single IP injection of xenogeneic human PBL antigen on day 0 changes the XA reactivity pattern in untreated LEW sera. Expectedly, XA production strongly increases and switches from IgM to IgG (IgG titers range from 1:1024 to 1:16384; IgM titers range from 1:4 to 1:256). IgG titers remain stable over the observation period (up to day +50), while IgM titers continuously decrease after day +10.

LF Treatment of Sensitized Rats

Treatment of xenogeneically sensitized LEW rats with 3 or 10 mg/kg LF from day -4 to day +10 inhibits both IgG and IgM XA production in a dose-dependent manner (Fig 1). This significant suppression of B-lymphocyte activation is maintained only during the period of LF application. Twenty days after LF has been withdrawn, IgG and IgM titers increase and resemble the untreated sensitized controls.

DISCUSSION

The isoxazol derivative LF, a novel immunosuppressant, is thought to antagonize cytokine activity and thus to interfere with T-helper cell-dependent B- and T-lymphocyte proliferation.² Its inhibitory effects on various experimental autoimmune diseases originally marked LF a strong inhibitor of B-cell activation.³ This effect led us to

study the potential of LF to suppress NXA- and XA-specific B-cell activation.

In spite of low serum titers, cytotoxic NXA are considered to be the main cause of hyperacute xenograft rejection in discordant combinations and are known to be very therapy resistant.⁴ It is evident from our studies that when LF is applied permanently it is capable of significantly inhibiting the renewed production of both IgM and IgG NXA in nonsensitized rats. More interestingly, the efficacy of LF increases when rat B lymphocytes, which are specific for XA, are activated by human xenoantigen (Fig 1). The activity of these cells is apparently suppressed for the duration of drug treatment. LF is also tolerated well. It therefore appears to be an attractive alternative to efforts that combine NXA- or XA-depleting procedures, for example, plasmapheresis, with conventional cytotoxic drug therapy, for example, cyclophosphamide.⁵

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