Pharmacokinetics and Immunological Effects of Exogenously Administered Recombinant Human B Lymphocyte Stimulator (BLyS) in Mice

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Abstract

B lymphocyte stimulator (BLyS; also known as TNFSF20, BAFF, TALL-1, zTNF4, and THANK), a tumor necrosis factor ligand family member, has recently been identified as a factor that promotes expansion and differentiation of the B cell population, leading to increases in serum immunoglobulin levels. Here, pharmacokinetic parameters for BLyS administered i.v. and s.c. to mice are described, and the effects of different dosing regimens on serum and salivary immunoglobulin levels as well as splenic cell populations are reported. The pharmacokinetics of BLyS following i.v. injection are monophasic with a half-life of 160 min, a clearance of 0.22 ml/min-kg, and a volume of distribution of 53 ml/kg. Systemic administration of BLyS to mice resulted in increased serum IgG, IgA, IgM, and IgE and salivary IgA as well as splenic cell population expansion and differentiation. The i.v. and s.c. routes of administration were pharmacologically equivalent, even though s.c. bioavailability of BLyS is only 25%. BLyS (s.c.) dramatically elevated serum IgG and IgA levels, and the duration of the responses after cessation of treatment (t1/2 = 4.4 and 1.3 days, respectively) are similar to the half-lives of endogenous IgG and IgA in mice. The IgM response is more modest than that of IgG and IgA but lasts longer (t1/2 = 7.0 days) than the half-life of endogenous IgM. A linear pharmacodynamic response was identified between days of dosing × log(dose), and increases in serum IgG, IgA, and IgM indicating that the response is more sensitive to the duration of dosing than to the cumulative dose. The implications of these findings for therapeutic administration of BLyS are discussed.

Members of the tumor necrosis factor (TNF) ligand superfamily of proteins participate in the regulation of immune function by modulating the physiologic processes of cellular proliferation, differentiation, survival, and apoptosis. These processes underlie the development of lymphoid tissue, normal hematopoiesis, inflammatory responses, activation and attenuation of normal immune responses, and removal of autoreactive T and B lymphocytes. B lymphocyte stimulator (BLyS, also known as TNFSF20, BAFF, TALL-1, zTNF4, and THANK), a TNF ligand family member, has recently been identified as a factor that promotes expansion and differentiation of the B cell population, leading to increases in serum immunoglobulin levels (Moore et al., 1999; Mukhopadhyay et al., 1999; Schneider et al., 1999; Shu et al., 1999; Tribouley et al., 1999).

Full-length BLyS is a 285-amino acid, type II transmembrane protein found in cells of myeloid origin, particularly those of monocytic lineage (Moore et al., 1999). BLyS shares homology with other members of the TNF ligand family, including APRIL, TNFα, lymphotoxin-α, and TRAIL. The highest levels of full-length BLyS message have been found in the spleen, lymph nodes, bone marrow, peripheral blood mononuclear cells, and myeloid cell lines (Moore et al., 1999). In a process similar to other TNF ligands, the membrane-bound version of BLyS is cleaved to form a soluble product upon stimulation of monocytic cells (Moore et al., 1999; Tribouley et al., 1999). A soluble form of BLyS consisting of 152 amino acids has been identified, isolated, and purified from mammalian and insect cells transfected with BLyS cDNA taken from a human neutrophil-monocyte library (Moore et al., 1999).

Two putative BLyS receptors have recently been identified: transmembrane activator and calcium-modulating cyclophilin ligand (CAML), otherwise known as TACI, and B cell maturation antigen (BCMA) (Gross et al., 2000). BCMA is expressed on B cells, whereas TACI is found on both B and Jurkat T cells (Gross et al., 2000). As predicted from its receptor distribution, BLyS has potent and selective mito-
genic activity on human and mouse B cells. BLyS increases supernatant immunoglobulin levels from human B cells cocultured with phytohemagglutinin/phorbol 12-myristate 13-acetate-stimulated T cells over that obtained with cytokines alone (Schneider et al., 1999). Administration of BLyS in mice increases the number of splenic B cell zones and elevates the number of CD45R+/ThB(Ly6D)+ B cells (Moore et al., 1999), a phenotype characteristic of terminally differentiated plasma cells (Hilbert et al., 1995). In addition, BLyS increases serum IgA and IgM (Moore et al., 1999). Further evidence of the involvement of BLyS in stimulation of B cell growth and differentiation into plasma cells comes from BLyS transgenic mice. These animals express large numbers of B cells and high levels of circulating immunoglobulins (Mackay et al., 1999; Gross et al., 2000; Khare et al., 2000). Aberrant continuous overexpression of BLyS in these animals leads to the development of a systemic lupus erythematosis-like syndrome characterized by enlargement of secondary lymphoid tissues, the generation of autoantibodies to nuclear antigens, proteinuria, and immune complex deposition in the kidney (Khare et al., 2000).

The identification of soluble BLyS may have therapeutic applications in B cell-related disease states such as isolated IgA, IgG, and IgM deficiencies, common variable immunodeficiency and other panhypogammaglobulinemias, and X-linked immunodeficiency with increased IgM. In these conditions, abnormal B cell function leads to global or specific hypogammaglobulinemia and/or decreased secretory immunoglobulin levels. In many of these disease states, the treatment of choice is parenteral administration of immunoglobulin. However, B cell expansion and differentiation by means of BLyS treatment could provide a unique alternative to conventional parenteral immunoglobulin therapy.

The development of BLyS for use in treating certain hypogammaglobulinemias has necessitated a better understanding of the pharmacokinetic and pharmacodynamic relationships of systemically administered BLyS. Here, pharmacokinetic parameters associated with i.v. and s.c. administered BLyS are described. The effects of different dosing regimens on serum and salivary immunoglobulin levels as well as splenic cell populations in the mouse are reported, and a pharmacodynamic model that describes these data is presented. Furthermore, the duration of response after the end of therapy is investigated and compared with reported half-lives of endogenous immunoglobulins. The implication of these findings for therapeutic administration of BLyS are discussed.

Animal Experimentation. All animal experimentation was done in accordance with the Guide for the Care and Use of Laboratory Animals and under the supervision of the Human Genome Sciences, Inc. Institutional Animal Care and Use Committee.

Pharmacokinetic Experiments. BALB/c mice, obtained from Ace Animals Inc. (Boyertown, PA), were allowed to acclimate for at least 1 week before the start of experiments. Male mice were from 7 to 9 weeks old and weighed 19 to 26 g. Female mice were 13 weeks old and weighed 18 to 21 g.

Mice in the i.v. groups were given BLyS or vehicle by injection via the tail vein at a dose of 0.3 mg/kg. Mice in the s.c. groups were given BLyS or vehicle by s.c. injection in the mid-scapular region at a dose of either 0.3 or 3.0 mg/kg. The stock solution of BLyS was diluted to 1.0 mg/ml with a 125 mM NaCl solution, pH 6.5. Further dilutions of BLyS to 0.3 and 0.03 mg/ml were made using a 12.5 mM citrate/125 mM NaCl buffer as the diluting solution. The vehicle control for the i.v. and s.c. groups consisted of 12.5 mM sodium citrate/125 mM NaCl buffer. The following plasma sampling schedule was used for mice that received BLyS via i.v. injection: 5, 30, 60, 180, 360, and 1440 min postinjection. The plasma sampling schedule for animals that received BLyS via s.c. injection was as follows: 30, 60, 180, 360, 960, 1440, and 2880 min postinjection. Four animals were injected for each time point in the i.v. and s.c. plasma sampling schedules. The mice were euthanized by CO₂ asphyxiation at the times listed above, and blood was drawn from the inferior vena cava into a syringe containing an EDTA solution. The blood was then injected into EDTA-coated microtainers, gently shaken, transferred to serum separator tubes, and centrifuged at 11,000 rpm for 5 min at 4°C. The plasma was separated and stored at −80°C for later analysis by enzyme-linked immunosorbent assay (ELISA).

To determine BLyS levels in the plasma, each sample was assayed in a solid phase, sandwich ELISA. The ELISA utilizes a murine monoclonal anti-BLyS antibody for capture and a biotinylated rabbit polyclonal antibody along with streptavidin-horseradish peroxidase for detection. Tetramethylbenzene was used as the peroxidase substrate. The optical densities of the plate wells were read at 450 nm in a Spectromax ELISA plate reader (Molecular Devices, Menlo Park, CA). The limit of detection of the ELISA is approximately 800 pg/ml.

The i.v. data were fit with a one-compartment model using the software package WinNonlin (Pharsight Corp., Mountain View, CA). s.c. data were fit with a one-compartment, first-order absorption model. When fitting the s.c. data, plasma concentrations from only the first 24 h were used, because <2% of BLyS was eliminated between 24 and 48 h. This method gave the best fit to the s.c. data for the period over which the majority of elimination occurs. Moreover, the plasma concentrations measured for both the 0.3 and 3.0 mg/kg, s.c., doses at 48 h were not statistically different from each other or from controls.

BLyS-Induced Changes in Salivary and Serum Immunoglobulins and Effects of Route of Administration. Seventy-two female BALB/c mice of similar age were randomized into 12 groups of 6 animals each. Mice were dosed daily with 0.3 or 3.0 mg/kg BLyS or vehicle (10 μl/g) administered either s.c. or i.v. (tail vein) for 4 (experimental days 1–4, inclusive) or 14 days (experimental days 1–14, inclusive) as outlined in Table 1.

On days 5 and 15 of the study, mice from groups 1 through 6 and 7 through 12, respectively, were dosed with 2.0 mg/kg pilocarpine i.p. and anesthetized with 60 mg/kg sodium pentobarbital (Veterinary Laboratories Inc., Lenexa, KS) i.p. then placed on a warming pad set for 37°C. For saliva collection, one end of a 10-cm piece of PE-90 tubing was inserted into the mouth of each mouse. The other end of the PE-90 tubing was pushed over a 21-gauge stainless steel tube and inserted into the septum of a Vacutainer (Becton-Dickinson, Franklin Lakes, NJ) tube containing no additive. The Vacutainer tube was placed under negative pressure using a common laboratory vacuum system. Saliva was suctioned into the Vacutainer tube for 20 min following pilocarpine challenge. The collected saliva volume was determined using an analytical balance by comparing tube weights...
TABLE 1
BlyS dosing schedule for determining the effects of route on serum and salivary immunoglobulin concentration

<table>
<thead>
<tr>
<th>Group</th>
<th>Route</th>
<th>BlyS Dose</th>
<th>Dosing Day</th>
<th>Necropsy Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s.c.</td>
<td>Vehicle</td>
<td>1, 2, 3, 4</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>i.v.</td>
<td>Vehicle</td>
<td>1, 2, 3, 4</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>s.c.</td>
<td>0.3</td>
<td>1, 2, 3, 4</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>i.v.</td>
<td>0.3</td>
<td>1, 2, 3, 4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>s.c.</td>
<td>3.0</td>
<td>1, 2, 3, 4</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>i.v.</td>
<td>3.0</td>
<td>1, 2, 3, 4</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>s.c.</td>
<td>Vehicle</td>
<td>1–14</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>i.v.</td>
<td>Vehicle</td>
<td>1–14</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>s.c.</td>
<td>0.3</td>
<td>1–14</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>i.v.</td>
<td>0.3</td>
<td>1–14</td>
<td>15</td>
</tr>
<tr>
<td>11</td>
<td>s.c.</td>
<td>3.0</td>
<td>1–14</td>
<td>15</td>
</tr>
<tr>
<td>12</td>
<td>i.v.</td>
<td>3.0</td>
<td>1–14</td>
<td>15</td>
</tr>
</tbody>
</table>

BlyS-induced Changes in Salivary and Serum Immunoglobulins and Effects of Route of Administration.

In general, s.c. or i.v. BlyS administration in mice increased serum IgG, IgA, IgM, and IgE levels over vehicle controls (Fig. 2, A–D), and at least 3 days of daily dosing are required to see these increases (data not shown). Although BlyS-induced serum IgG, IgA, and IgM increases were not dependent on route of administration, the duration of administration played a significant role in the magnitude of this

Results

Pharmacokinetics of BlyS. BlyS plasma concentrations following i.v. injection of 0.3 mg/kg BlyS in both male and female mice declined monoeXponentially over a 24-h period (Fig. 1A). A one-compartment model was used to analyze the data, and the corresponding pharmacokinetic parameters are summarized in Table 3. A two-way ANOVA of the concentration data comparing males and females across all time points indicates no significant difference between the two groups. The half-life of BlyS following i.v. injection in mice was approximately 160 min. The clearance was 0.21 to 0.24 ml/min-kg. The volume of distribution for BlyS was approximately 53 ml/kg, or slightly greater than the plasma volume.

Plasma concentrations following s.c. injection of 0.3 and 3.0 mg/kg BlyS in male mice are shown in Fig. 1B. Pharmacokinetic parameters for the two doses are summarized in Table 3. The volume of distribution, clearance, and elimination half-life of BlyS given by s.c. injection are not dependent on dose over the range tested (i.e., Cmax and area under the concentration curve are proportional to the dose administered). The elimination half-life of BlyS following s.c. injection was approximately 160 min, the same as following i.v. injection. The bioavailability of BlyS following s.c. injection was 23 to 26%.

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response (Fig. 2, A–C). With regard to specific immunoglobulin responses, i.v. and s.c. BLyS administration caused significant 2- to 3-fold increases in serum IgG and IgA with 4 days of dosing (Fig. 2, A and B). IgM was not significantly elevated by 4 days of s.c. or i.v. dosing. The magnitude of the i.v. or s.c. BLyS-induced increase in serum IgG, IgA, and IgM was substantially greater with 14 consecutive days compared with 4 days of once daily dosing. Specifically, IgG levels increased by 4- to 8-fold, IgA levels by about 6- to 10-fold, and IgM levels by 2-fold over baseline (Fig. 2, A, B, and C, respectively). With 14 days of dosing, the increase in serum immunoglobulin was found to be maximal at the doses tested in terms of the IgM response; however, the IgA and especially the IgG responses appeared dose-dependent.

The effects of i.v. and s.c. BLyS administration on serum IgE levels were somewhat different than those seen for the other immunoglobulins. Specifically, i.v. BLyS administration had no significant effect on serum IgE at either dose or duration of administration tested compared with vehicle controls (Fig. 2D). However, s.c. BLyS administration produced a small but significant increase in serum IgE in the 3.0 mg/kg dose group after 4 days of administration and a robust and dose-dependent increase after 14 days of administration.

The effects of s.c. and i.v. administration of BLyS on salivary IgA in pilocarpine-treated mice are shown in Fig. 3. Although serum IgA levels were elevated by about 3-fold with 4 consecutive daily doses of 0.3 or 3.0 mg/kg BLyS, salivary IgA levels were not significantly different from vehicle controls with either s.c. or i.v. administration after 4 consecutive days of dosing at any dose tested. However, a significant 2.5-fold increase in salivary IgA was noted in animals treated with 3.0 mg/kg BLyS by either the i.v. or s.c. routes after 14 consecutive daily doses.

An empirical pharmacodynamic model was developed to describe the serum IgG, IgA, and IgM responses. Although pharmacokinetic experiments showed only a 23 to 26% bioavailability from s.c. injection, i.v. and s.c. injections elicited the same level of IgG, IgA, and IgM elevation at both doses (0.3 and 3.0 mg/kg) and both dosing periods (4 or 14 days). Therefore, i.v. and s.c. data were combined when exploring the pharmacodynamics. BLyS given for longer dosing periods elicited a greater response, as did higher doses of BLyS for the IgG and IgA responses. For example, 0.3 mg/kg given for 14 days (cumulative dose = 4.2 mg/kg) stimulated a greater response than 3.0 mg/kg given for 4 days (cumulative dose = 12 mg/kg). To explore the relationship among duration of dosing, daily dose, and immunoglobulin response, several transformations of these variables were examined. It was found that when days of dosing × log(dose) was used as the dependent variable, the changes in serum IgG, IgA, and IgM levels in BLyS-treated mice relative to vehicle-treated controls demonstrated a linear pharmacodynamic response with a positive slope (Fig. 4).

Duration of BLyS-Induced Effects. Preliminary experiments showed that serum immunoglobulins returned to baseline by 1 to 2 weeks after daily BLyS injections were terminated (data not shown). A more detailed evaluation of the time course of recovery following 10 days of dosing was undertaken, and the results are shown in Fig. 5. BLyS elicited a strong IgG response: 6 h after the last BLyS injection, endogenous IgG levels were ~3.5 times higher than in control mice (Fig. 5A). IgG levels declined over the next 12 days, but for 9 days they remained higher than IgG levels in control mice. However, by 12 days, there was no statistically significant difference between the two groups. BLyS also stimulated a robust IgA response—endogenous IgA levels in BLyS-treated animals were 6 times those in controls at 6 h after the last BLyS injection (Fig. 5B). However, the duration of the elevated IgA levels was short-lived; 3 days after the
last BLyS injection, IgA levels were only twice that of controls, and by 6 days, there was no significant difference between the BLyS-treated and control groups. BLyS stimulated a more modest IgM elevation, but the effect appears to be the longest in duration compared with IgG and IgA (Fig. 5C). IgM levels were 65% higher in the BLyS-treated group than in the control group 6 h after the last BLyS injection. IgM levels in BLyS-treated animals remained significantly higher than vehicle controls through day 12 ($p < 0.001$) when IgM levels were 33% higher than the control group.

The difference between immunoglobulin levels in BLyS-treated and control mice after termination of BLyS treatment is plotted in Fig. 5D. The difference appeared to follow a first-order rate of decline. The half-lives corresponding to the recovery of the IgG, IgA, and IgM responses were 4.4, 1.3, and 7.0 days, respectively.

**BLyS Effects on Spleen Weight.** In a separate study, mice treated s.c. with 0.3 or 3.0 mg/kg/day BLyS for 3 or 4 days and euthanized on day 4 showed a 40 to 55% increase in spleen weight (Fig. 6A). In animals treated s.c. with 3 mg/kg/day BLyS for 15 days and euthanized on day 16, the magnitude of the increase in spleen weight was approximately 60% compared with vehicle control (Fig. 6B; note, the 0.3 mg/kg dose level was not tested in the 16-day paradigm). Interestingly, spleen weights were not elevated in animals treated s.c. with 3.0 mg/kg/day BLyS for 2, 4, or 9 days and euthanized on day 16 when compared with animals receiving vehicle. This indicates that the BLyS-induced increase in splenic weight is reversible.

**BLyS Effects on Splenic Cell Populations.** FACS analyses of the BLyS and vehicle group splenocytes revealed a general trend toward BLyS-induced expansion in the relative number of more developmentally mature splenic B cells ($B_{220}^+$/$Th_{B}^+$) compared with time-matched vehicle controls (Fig. 7, A–C). This increase was observed in animals given a single dose of BLyS on day 1 and euthanized on day 5. In animals treated with BLyS for 2, 4, or 9 days and euthanized on day 16, no changes in the relative number of mature splenic B cells were noted. However, with 15 days of dosing, the relative number of mature splenic B cells was elevated compared with vehicle-treated animals euthanized on day 16. The lack of a sustained change in the relative number of mature splenic B cells after even 9 days of dosing in animals necropsied on day 16 suggests that this effect is reversible. Interestingly, a small decrease in the relative number of $B_{220}^+$/ThB− cells was observed in animals treated for either 4 or 15 days in the 5- and 16-day groups, respectively (data not shown). One possible explanation for this effect is that BLyS may cause a shift from these less mature B cells ($B_{220}^+$/ThB−) toward a more mature state ($B_{220}^+$/ThB+).
A reduction of approximately 25% of CD8+ spleen cells was also observed compared with time-matched vehicle-treated animals (data not shown). This effect was observed in the animals necropsied on day 5 following 2, 3, or 4 days of dosing, but only after 15 days of dosing in animals necropsied on day 16. The T cell results again suggest the reversibility of BLyS effects. No other consistent treatment-related changes were observed in other splenic cell populations.

Discussion

BLyS, a growth factor that promotes B cell proliferation and differentiation, is being developed to increase the production of endogenous immunoglobulins in patients with certain hypogammaglobulinemias. To assist in developing a dosing plan for a clinical trial of BLyS, the current study examined pharmacokinetic parameters of s.c. and i.v. administered BLyS, the effects of BLyS on serum and salivary immunoglobulins and splenic cell populations, and the relationships between BLyS pharmacokinetics and its pharmacologic effect in mice.

Systemic administration of BlyS to mice resulted in increases in serum IgG, IgA, IgM, and IgE and salivary IgA. The serum immunoglobulin level increases following BLyS administration are dependent on the duration of dosing in that increases in serum immunoglobulins were substantially greater following 14 to 15 days of dosing compared with just 4 days of dosing. It should be noted that, since dosing was not continued past 15 consecutive days, the maximum possible increase in the levels of serum immunoglobulins at a particular dose is not known. Intravenous and s.c. routes of administration were equally effective in stimulating increases in serum IgG, IgA, and IgM, whereas only the s.c. route was effective at raising IgE levels. With regard to mucosal immunoglobulins, BLyS increased salivary IgA with a delayed kinetic profile compared with its effects on serum IgA. This observation is consistent with stimulation of salivary IgA secretion by normal physiologic mechanisms of mucosal B-cell proliferation and trafficking (Delacroix et al., 1982). Increases in both serum and salivary immunoglobulins appear to be reversible, since levels returned to baseline by at least 1 week after the cessation of dosing.

Administration of BLyS also resulted in a trend toward splenic B cell population expansion and differentiation (relative number and progression to a more mature form). This effect was observed in mice euthanized after up to 4 days of dosing even when only a single dose of BLyS was administered. Interestingly, the magnitude of the effect of BLyS on spleen weight or relative B cell number was not substantially greater after 2 weeks of dosing compared with four consecutive daily doses in animals euthanized 1 day after the last dose was administered. The rapidity of the splenic B cell response compared with the delayed increase in the concentration of serum immunoglobulins suggests that the mechanism for BLyS-induced increases in serum immunoglobulins is mediated by B-cell expansion/differentiation rather than stimulation of pre-existing plasma cell immunoglobulin secretion (Moore et al., 1999; Schneider et al., 1999). As seen with the serum and salivary immunoglobulin responses, splenic B cell changes as well as increases in spleen weight returned to basal levels by at least 1 week after the cessation of dosing.

Although BLyS is a member of the TNF superfamily of proteins, its rate of clearance, 0.21 to 0.24 ml/min-kg, is more than 10 times lower than other TNF-like proteins. For example, recombinant human TNF-α was found to have a clearance of 2.4 to 3.0 ml/min-kg in mice (Ferraiolo et al., 1988). Similarly, human leucine zipper TNF-related apoptosis-in-
Producing ligand (TRAIL) has a clearance of 2.9 ml/min-kg in mice based on data from Walczak et al. (1999). The mechanism for the slower clearance of BLyS compared with related proteins is unknown, although its very anionic state (pI 4.7) may contribute to retarded filtration at the glomerular membrane (Deen et al., 1983). The existence of a BLyS-binding protein in plasma offers another explanation worthy of further investigation. The lack of dose dependence on clearance argues against, but does not rule out, this possibility.

The effects of BLyS on serum immunoglobulins do not appear to be directly proportional to the plasma concentration of BLyS. This is demonstrated by the observation that the s.c. and i.v. routes of BLyS administration were equally effective at increasing serum IgG, IgA, and IgM levels, even though the bioavailability of BLyS following s.c. injection is only 23 to 26%. Because B cells, the target cells for BLyS, are found predominantly in lymphoid tissues, BLyS concentrations in those tissues may be more closely correlated to efficacy than plasma concentration. Subcutaneously injected proteins with molecular masses greater than 16 kDa are taken up mainly via the lymphatic system and transit through lymph nodes before entering the bloodstream (Guyton, 1981; Supersaxo et al., 1990; Porter and Charman, 2000). Thus, there may be significant exposure of BLyS (mo-

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**Fig. 5.** Duration of s.c. BLyS-induced effects on serum immunoglobulin levels after treatment is terminated. A, IgG; B, IgA; C, IgM; and D, rate of decay of serum immunoglobulin responses. Data are reported as mean serum immunoglobulin concentrations ± S.E.M. n = 4–5 per treatment group, *p < 0.05.

**Fig. 6.** Effect of s.c. BLyS administration on spleen weight. A, assessment at day 5 for up to 4 days of dosing; B, assessment at day 16 for up to 15 days of dosing. Data are mean spleen weight ± S.E.M. n = 4 per treatment group, *p < 0.05.
molecular mass = 46 kDa) to lymphoid tissues when injected via the s.c. route even though only 23 to 26% is eventually absorbed into the circulation. The balance of the injected dose may be cleared via the lymphatics and lymphoid tissues or degraded before reaching the bloodstream (Porter and Charman, 2000). If the exposure of BLyS to lymphoid tissues is similar following both i.v. and s.c. injection, equivalent B-cell-mediated responses should be achieved via both routes despite the low apparent bioavailability measured from plasma monitoring. In addition, preliminary biodistribution studies with radioiodinated BLyS (data not shown) suggest that BLyS rapidly and specifically localizes to lymphoid tissues and is cleared from those tissues more slowly than from plasma. A longer half-life of BLyS in lymphoid tissues may account for the observation that once daily dosing in mice was sufficient to elicit a response even though the half-life in plasma is only 160 min. This further supports the hypothesis that the pharmacodynamic effects of BLyS are a function of lymphoid exposure, as governed by lymphatic uptake and binding to lymphoid tissues, rather than plasma concentration.

Dose-response relationships often demonstrate linearity between 20 and 80% of the effect level when the log of the dose or the log of the cumulative dose is compared with the pharmacological effect. However, with BLyS it was found that increases in serum IgG, IgA, and IgM were stimulated more strongly by a low dose given for a long dosing period than a high dose given for a short dosing period. To search for a possible BLyS dose-response relationship that incorporated the importance of the duration of dosing, several different transformations of the data were assessed. Linearity was found between increases in serum IgG, IgA, and IgM and the product of duration and log(dose). The relatively greater sensitivity of the immunoglobulin response to duration of dosing than to the cumulative or daily dose is not unexpected for a protein that exerts its effect through a saturable receptor.
Injected BLyS stimulates different serum immunoglobulin classes to different degrees, and the duration of the responses also varies. IgA levels are stimulated to the greatest extent, but the duration of the response after treatment ends is also very short-lived. The half-life of the stimulated IgA response (1.3 days) is comparable to the half-life of endogenous IgA of approximately 1.0 day (Vieira and Rajewsky, 1988). It thus appears that, after treatment is stopped, stimulation of IgA production quickly reverts to normal levels, and the duration of elevated levels largely represents the normal rate of clearance of IgA. IgG is also strongly stimulated, although to a lesser extent than IgA. The duration of the response (t_{1/2} = 4.4 days) is similar to the half-life of endogenous IgG of 4.0 to 5.4 days in a mouse (Waldmann and Strober, 1969). Thus, it appears that BLyS-induced production of IgG, like that of IgA, quickly reverts to normal after treatment is terminated. On the other hand, the IgM response is more modest but much longer-lived (t_{1/2} = 7.0 days) than the endogenous half-life of IgM in a mouse [0.2–0.6 days (Waldmann and Strober, 1969), 2 days (Vieira and Rajewsky, 1988)]. The hypothesis is that, since all B cells proceed through the IgM isotype, withdrawal of BLyS stimulation results in a decrease in the number of stimulated B cells undergoing isotype switching and terminal differentiation into plasma cells of the IgG and IgA isotypes. This scenario would produce a transient relative overabundance of IgM-producing B cells. The more persistent stimulation of IgM production merits further investigation of the differential effects of BLyS on different classes of B cells.

In summary, BLyS administered i.v. or s.c. increases B cell proliferation and differentiation, followed by increases in the production of serum IgG, IgA, and IgM. These increases in serum immunoglobulin levels provide a useful means of monitoring the efficacy of BLyS in the clinic. Although s.c. bioavailability of BLyS was only 25%, effects on IgG, IgA, and IgM were similar by either the i.v. or s.c. route. Multiple daily doses of BLyS were required to elicit consistent effects in all the measured immunological endpoints induced by BLyS. After dosing was stopped, serum IgG and IgA levels returned to baseline values at a rate consistent with the normal clearance rates of these immunoglobulins, whereas IgM levels normalized more slowly. BLyS effects on salivary immunoglobulin levels, spleen weight, and B cell differentiation in mice were also reversible, normalizing as early as 1 week after cessation of dosing. These results provide important information for the design of a clinical dosing plan for BLyS and demonstrate that BLyS may offer enhanced host immunological protection in treating certain hypogammaglobulinemias without the need for intravenous immunoglobulin G.

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References


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