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Modulation of a Human Immunosuppressive Lymphokine by Monosaccharides[1]

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Soluble suppressor factor (SSF) is a recently purified human lymphokine produced by peripheral blood lymphocytes (PBL) in serum-free medium as a likely consequence of an autologous mixed lymphocyte reaction. Immunoregulatory actions of SSF include suppression of: polyclonal B cell activation, proliferative responses of normal PBL, and natural killer (NK) and antibody-dependent cellular cytotoxicity. We examined the ability of the monosaccharides fucose (Fuc), galactose (Gal), glucose (Glc), and mannose (Man) to reverse SSF-mediated suppression of NK activity. Fuc and Gal can partially or completely reverse SSF-mediated suppression at four effector:target cell ratios. Man and Glc were unable to significantly reverse SSF-mediated suppression. Fuc or Gal was added to PBL at various times after addition of SSF. SSF-mediated suppression of NK cytotoxicity becomes irreversible with respect to these monosaccharides during the first 24 hr of PBL exposure to SSF. To explore the mechanism behind this block of SSF-mediated suppression, Fuc or Gal (50 m*M*) was cultured with PBL for 24 hr before addition of SSF, or with SSF for 24 hr before addition to PBL. Our experiments indicate that SSF is directly interacting with these monosaccharides, and may function by recognizing specific sugar moieties on the surface of effector cells.

INTRODUCTION

Soluble suppressor factor (SSF) is a heat-stable protein lymphokine produced by peripheral blood lymphocytes (PBL) in serum-free medium (1). SSF induction is apparently a consequence of the autologous mixed lymphocyte reaction, as it is produced by neither T cells nor B cells alone. Biological actions of SSF include suppression of polyclonal B cell activation, proliferative responses of normal PBL, and natural killer (NK) and antibody-dependent cellular cytotoxicity.

We have partially completed the biochemical characterization of SSF. This lymphokine appears to have a molecular weight of approximately 17 kDa as determined by gel filtration and membrane ultrafiltration (2). SSF is heat stable at 56°C for 30 min, but loss of suppressor activity occurs after incubation at 70°C for 30 min (3). SSF is sensitive to proteolytic enzymes, suggesting that it is, at least in part, a polypeptide (1).

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We have recently purified SSF by a combination of controlled-pore glass chromatography, polyacrylamide gel electrophoresis, and isoelectric focusing gel electrophoresis (4). The purified material contains two isoforms with pI values of 8.2 and 8.4. Both isoforms mediate suppression of NK cytotoxicity, and suppression by either can be inhibited by anti-SSF mAb. It is unknown whether one isoform is an artifact produced from native SSF by the harsh purification process, or both forms are produced as native SSF.

The specific lymphocyte subpopulation responsible for the production of SSF has not yet been completely determined; however, preliminary studies indicate that the cell producing SSF is a $CD3^+$, $CD4^-$, $CD8^+$, HNK-1^+ lymphocyte. Production of SSF is unaffected by preirradiation of lymphocytes with up to 2000 rads (1), suggesting that SSF may be produced by a unique radioresistant subset of lymphocytes of the suppressor phenotype.

Interferon-α (IFN) and SSF have been shown to have reciprocal effects on NK cells. Suppression of NK cytotoxicity mediated by SSF can be partially reversed by incubating effector cells with IFN (3). We have recently shown that IFN can also stimulate the production of SSF, suggesting that these two lymphokines regulate a balance of immune suppression and stimulation (5).

The role of monosaccharide-containing moieties in lymphokine–leukocyte interactions is currently a topic of considerable interest. Interactions between SSF and specific monosaccharides have been briefly described (3). In this study, we expand upon these preliminary observations by examining the ability of a broad range of monosaccharides to reverse SSF-mediated suppression of NK cytotoxicity. We also describe the mechanism of binding of SSF to target cells, and determine the time course of SSF-mediated suppression induced by this binding.

MATERIALS AND METHODS

Isolation of cells. Heparinized blood (20 U/ml) was obtained from a peripheral vein of healthy subject donors and diluted with an equal volume of pH 7.4 phosphate-buffered saline (PBS). The mononuclear cell fraction was isolated by density gradient centrifugation through a solution of sodium metrizoate and Ficoll (Lymphoprep, Nygaard & Co., A/S, Oslo, Norway) at 400*g* for 30 min at ambient temperature. The mononuclear cell band was harvested, washed 3 times with PBS, and resuspended in RPMI 1640 medium containing 25 m*M* Hepes buffer (Whitaker Scientific, Walkerville, MD) supplemented with 80 μg/ml gentamicin (Schering Corp., Kenilworth, NJ) and 300 μg/ml fresh glutamine (complete medium).

Depletion of adherent cells. Peripheral blood mononuclear cells (PBMC) were depleted of adherent cells by passage through a Sephadex G-10 (Pharmacia Fine Chemicals, Piscataway, NJ) column. PBMC were suspended in complete medium supplemented with 10% heat-inactivated fetal calf serum (FCS) (GIBCO, Grand Island, NY) and passed through a 7-ml column of Sephadex G-10 beads equilibrated in the same medium. After 45 min incubation at 37°C, nonadherent cells were washed through with 2 vol of 37°C medium. Characteristically, cell recovery with this technique is >70% of the total input, and macrophage contamination of the resulting PBL, as indicated by nonspecific esterase stain, is <2% (6).

Preparation of SSF. Total PBMC or PBL, after 3 washes in PBS, were resuspended in serum-free RPMI 1640 medium containing only gentamicin and glutamine. Sus-

pensions of 5×10^6 cells/ml were incubated at 37°C in a humidified environment of 5% CO_2 in air for 1 day. After incubation, all cell suspensions were centrifuged at 500*g* for 10 min, washed with medium, and resuspended at 5×10^6 cells/ml in fresh RPMI 1640 medium containing only gentamicin and glutamine. These suspensions were incubated at 37°C in a humidified environment of 5% CO_2 in air for 3 additional days. After this incubation, supernates were separated by centrifugation at 500*g* for 10 min and stored at −20°C.

Incubation of effector cells with SSF. Duplicate cultures of 2×10^6 cells/ml in RPMI 1640 medium plus glutamine, gentamicin, 1 to 5% FCS v/v and mock SSF (serum-free medium) or SSF at a concentration of 20% v/v were incubated in a humidified environment of 5% CO_2 in air at 37°C for 72 hr. Cells were then washed twice and resuspended in complete medium. Treated and control cultures were tested for their ability to mediate NK activity versus the human erythroleukemic cell line, K-562, in a 4-hr ^{51}Cr release assay.

Preparation of target cells. K-562 cells were serially passed in complete medium until needed for analysis of NK activity. To complete medium containing K-562 cells, 100 μCi of ^{51}Cr as sodium chromate (New England Nuclear, Boston, MA) was added per 1×10^6 cells. The cells were incubated at 37°C for 1 hr in a humidified atmosphere of 5% CO_2 in air with intermittent shaking. After incubation, they were washed 3 times with complete medium and resuspended at 1×10^5 cells/ml in same.

Assay for NK activity. NK cytotoxicity was determined in a direct ^{51}Cr release assay as previously described (3). Variable numbers of viable effector cells in complete medium were added to triplicate cultures of 1×10^4 ^{51}Cr-labeled K-562 cells (effector: target ratios of 50:1, 20:1, 10:1, and 5:1) in final volumes of 0.2 ml in V-bottom microtitration plates (Costar, Cambridge, MA). After centrifugation at 40*g* for 5 min, they were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 4 hr. Microtitration plates were then centrifuged for 5 min at 400*g*, and 0.1-ml samples of supernate were removed from each well and measured for ^{51}Cr content.

Fucose affinity chromatography. Affinity chromatography of SSF was performed using L-fucose-linked agarose beads (Sigma Chemical Co., St. Louis, MO). Fucose-linked agarose beads were washed several times with 50 m*M* sodium phosphate, pH 6.8 (column buffer) and placed in a 10-ml plastic syringe. Loose beads were washed through by the addition of several volumes of column buffers. SSF was dialyzed against column buffer for 24 hr at 4°C, then applied to the affinity column. After 2 hr at room temperature, unbound material was washed through the column by the addition of several volumes of column buffer. The column was then incubated for 1 hr with each of three elution buffers, and specific elutant fractions were recovered by the addition of several volumes of the buffer. The elution buffers, in order of use, are: 1 *M* NaCl (pH 7.4), 4.5 *M* $MgCl_2$ (pH 7.5), and 3.5 *M* NaSCN (pH 8.0). Eluants from the column buffer wash and each elution buffer were concentrated using YM 5 (5-kDa molecular weight cut-off) ultrafiltration membranes (Amicon Corp., Danvers, MA). These concentrates were then tested for their ability to modulate NK cytotoxicity as described above, with cytotoxicity determined at E:T ratios of 25:1 and 50:1.

Calculations. Percentage cytotoxicity was calculated as follows:

$$\% \text{ cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100,$$

where spontaneous release represents counts released from control wells containing

only 1×10^4 labeled target cells and total release represents counts obtained from a lysate of 1×10^4 target cells.

Percentage suppression mediated by the addition of SSF-containing supernates was determined as follows:

$$\% \text{ suppression} = \frac{\% \text{ control cytotoxicity} - \% \text{ experimental cytotoxicity}}{\% \text{ control cytotoxicity}} \times 100.$$

Lytic units were calculated from percentage cytotoxicity values determined at varying effector cell:target cell ratios, as described by Kadish and co-workers (7). Data is expressed in terms of lytic units per 1×10^7 effector cells (LU). Percentage suppression of LU of NK activity was calculated as follows:

$$\% \text{ suppression} = \frac{(\text{LU of mock SSF-cultured lymphocytes}) - (\text{LU of SSF-cultured lymphocytes})}{\text{LU of mock SSF-cultured lymphocytes}} \times 100.$$

Statistics. Statistical significance was determined using the Wilcoxon Signed-Rank Test (8).

RESULTS

1. Effects of monosaccharides on natural killer cell cytotoxicity. PBL were cultured *in vitro* in the absence or presence of specific monosaccharides, at concentrations of 25 and 50 m*M*, for 3 days. NK cytotoxicity was then determined in a 4-hr ^{51}Cr release assay. Monosaccharide concentrations of 25 and 50 m*M* were chosen because these values represented possible physiological levels of monosaccharides, not pharmacologic values. Figure 1 shows the results of 11 separate experiments. The effects of six different monosaccharides, L-fucose (Fuc), D-galactose (Gal), D-glucose (Glc), D-mannose (Man), D-mannose-6-phosphate (6-MP), and *N*-acetyl-neuraminic acid (NANA), on NK cytotoxicity were examined. Fuc, Gal, Glc, and Man had no significant effect on NK cytotoxicity at either concentration tested. 6-MP caused statistically significant suppression ($P < 0.05$) of NK cytotoxicity at both concentrations tested. NANA also caused statistically significant suppression ($P < 0.05$) of NK cytotoxicity at a concentration of 50 m*M*, while the suppression at 25 m*M* was not statistically significant.

2. Effects of SSF on NK cytotoxicity in the absence or presence of monosaccharides. The ability of SSF to suppress NK cytotoxicity has been well established. It was thus examined whether certain monosaccharides could partially block this suppression. PBL were cultured *in vitro* for 3 days in the presence of 20% SSF-containing supernates with and without selected monosaccharides at concentrations of 25 or 50 m*M*. NK cytotoxicity was then determined at four effector:target cell (E:T) ratios. NANA and 6-MP were not used in this study, as their own suppressive effects would be difficult to differentiate from those of SSF. Figure 2 shows results from 17 separate experiments. SSF can cause statistically significant ($P < 0.01$) suppression of NK cytotoxicity at all four E:T ratios. However, culturing PBL with SSF and Gal or Fuc at either 25 or 50 m*M* resulted in significant reversal of SSF-mediated suppression of NK cytotoxicity at E:T ratios of 5:1, 10:1, and 20:1. At an E:T ratio of 50:1, significant reversal of SSF-mediated suppression resulted from culturing PBL with SSF and 25 or 50 m*M*

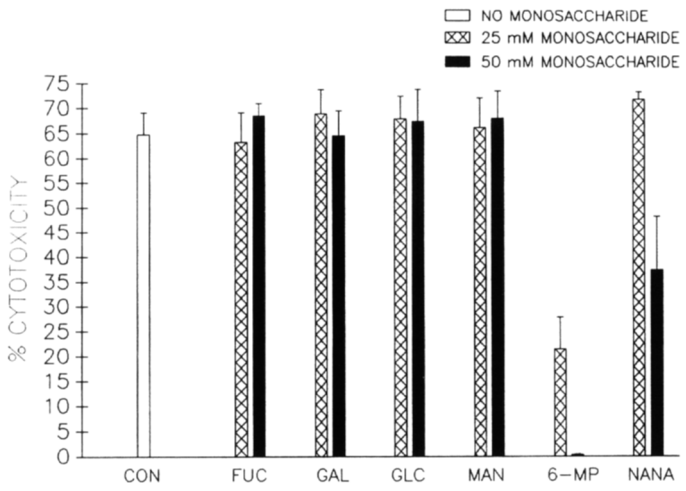

FIG. 1. Effect of monosaccharides on NK cytotoxicity. Fresh nonadherent PBL were incubated in RPMI 1640 medium supplemented with 1% fetal calf serum and 20% mock SSF (serum-free medium) in the absence or presence of monosaccharide (25 or 50 m*M*). After 3 days, the NK activity of these cells was determined against K562 targets at effector:target cell ratios of 50:1. Percentage cytotoxicity was calculated as described under Materials and Methods, and mean values are represented. The vertical lines represent the standard errors of the means. Difference between % cytotoxicity for cells cultured in the absence of monosaccharides (control) and % cytotoxicity for cells cultured with 50 m*M* M-6P is statistically significant at $P < 0.01$; the difference between control cells and cells cultured with 25 m*M* M-6P or 50 m*M* NANA is statistically significant at $P < 0.05$. No other differences in % cytotoxicity between control cells and cells cultured in the presence of monosaccharides are statistically significant.

Gal or with SSF and 50 m*M* Fuc. Reversal of SSF-mediated suppression by 25 m*M* Fuc was not statistically significant at the E:T ratio of 50:1. Culturing PBL with SSF and Glc or Man at both concentrations did not cause a statistically significant reversal of SSF-mediated suppression of NK cytotoxicity at any E:T ratio examined.

3. Stereochemical specificity of monosaccharide blockade of SSF-mediated suppression. The stereochemical structure of Fuc and Gal is identical; the only difference between these molecules is that C-6 of Fuc is dehydroxylated. To determine the specificity of this stereochemical configuration in reversing SSF-mediated suppression, the effects of L-Fuc and D-Fuc were compared. Results from four experiments are shown in Table 1. In a pattern similar to that of the results discussed above, neither L- nor D-Fuc at concentrations of 25 m*M* was able to significantly reverse SSF-mediated suppression at the E:T ratio of 50:1. However, L- and D-Fuc at concentrations of 50 m*M* showed similar results in significantly ($P < 0.05$) reversing SSF-mediated suppression at this E:T ratio. These results indicate that L- and D-Fuc are equally effective in reversing suppression mediated by SSF.

4. Site of action of Fuc and Gal blockade of SSF-mediated suppression. Three possible mechanisms by which Fuc and Gal could block SSF-mediated suppression of NK cytotoxicity include: (1) Fuc and Gal could interact directly with the SSF molecule, preventing it from binding to the cell surface; (2) Fuc and Gal could bind to a molecule on the effector cell surface, preventing subsequent binding of SSF; or (3) a combination of both of the above mechanisms. A set of experiments were performed to determine which of these possibilities was actually occurring. PBL were cultured for 3 days *in*

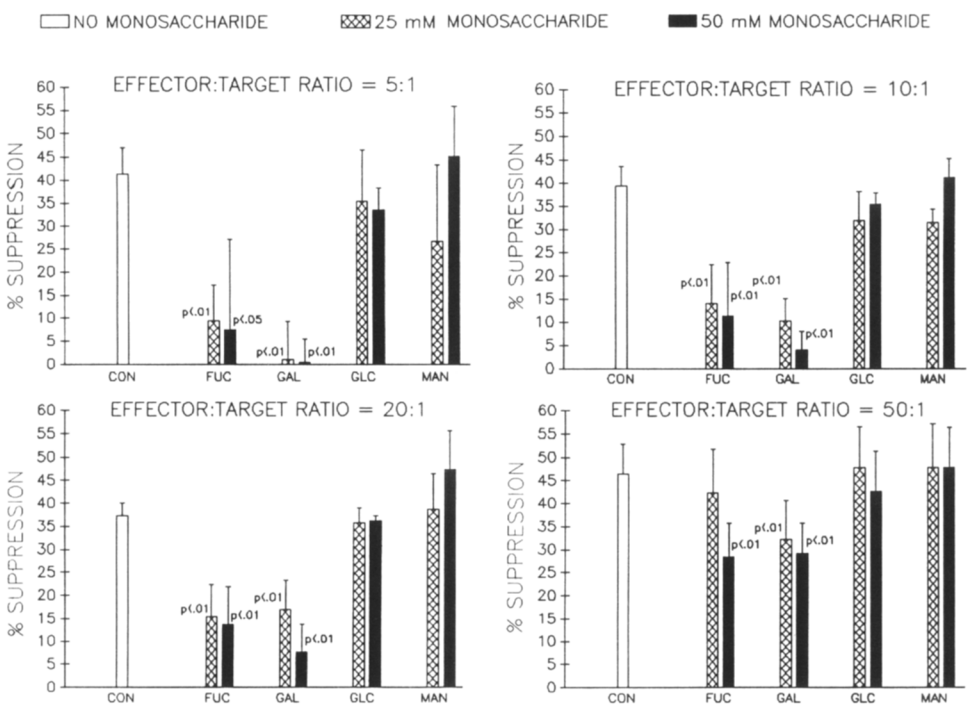

FIG. 2. Effect of monosaccharides on SSF-mediated suppression of NK cytotoxicity. Fresh nonadherent PBL were incubated in RPMI 1640 medium supplemented with 1% fetal calf serum and 20% SSF in the absence or presence of monosaccharide (25 or 50 m*M*). After 3 days the NK activity of these cells was determined against K562 targets at effector:target cell (E:T) ratios of 5:1, 10:1, 20:1, and 50:1. Percentage suppression was calculated as described under Materials and Methods, and mean values are represented. The vertical lines represent the standard errors of the means. The difference between % suppression for cells cultured in the absence of monosaccharides (SSF alone) and % suppression for cells cultured with SSF and monosaccharides are shown. No other differences in % suppression between cells cultured with SSF alone and cells cultured with SSF in the presence of monosaccharides are statistically significant.

vitro using one of the following protocols: (1) PBL were cultured alone for 24 hr, after which SSF and Fuc or Gal (50 m*M*) were added, and the mixture cultured for an additional 48 hr; (2) PBL were cultured alone for 24 hr while SSF was incubated with either Fuc or Gal (50 m*M*) in the absence of cells; after this period, the monosaccharide-SSF mixture was added to the PBL, and the mixture was cultured for an additional 48 hr; (3) PBL were cultured with only Fuc or Gal (50 m*M*) for 24 hr, after which SSF was added, and the mixture cultured for an additional 48 hr.

The results of this study from eight separate experiments are shown in Fig. 3. Again, SSF by itself was able to mediate significant suppression of NK cytotoxicity. Addition of Fuc or Gal simultaneous to the addition of SSF (Protocol 1) resulted in a statistically significant ($P < 0.05$) reversal of SSF-mediated suppression. Culturing of SSF with Fuc or Gal for 24 hr before addition to PBL (Protocol 2) similarly resulted in a significant ($P < 0.05$) reversal of SSF-mediated suppression. Finally, culturing PBL with Fuc or Gal for 24 hr before the addition of SSF (Protocol 3) resulted in no significant reversal of SSF-mediated suppression. These results suggest that in reversing SSF-mediated suppression of NK cytotoxicity, Fuc or Gal is interacting with the SSF moiety itself, and not with a binding site on the effector cell surface.

TABLE 1

Effects of L-Fuc and D-Fuc on SSF-Mediated Suppression of NK Cytotoxicity[a]

Condition	% Cytotoxicity[b]	P value vs control	P value vs SSF
Control	61.4 ± 1.8	—	—
SSF	34.9 ± 8.5	$P < 0.05$	—
SSF + 25 m*M* L-Fuc	36.4 ± 6.6	—	NS
SSF + 50 m*M* L-Fuc	49.5 ± 4.8	—	$P < 0.05$
SSF + 25 m*M* D-Fuc	37.8 ± 8.5	—	NS
SSF + 50 m*M* D-Fuc	49.3 ± 8.5	—	$P < 0.05$

[a] PBL were cultured for 3 days with mock SSF (control), SSF, or SSF plus the indicated monosaccharide as 20% supplements. NK activity of these cells was then determined as described under Materials and Methods at effector:target cell ratios of 50:1.

[b] Mean values ± standard errors of the means for four experiments are shown.

5. Kinetics of Fuc or Gal blockade of SSF-mediated suppression. It has been demonstrated that SSF can suppress NK cytotoxicity, and that Fuc or Gal, added at the same time as SSF, can partially reverse this suppression. The point at which SSF-mediated suppression becomes irreversible with respect to the addition of Fuc or Gal was next examined. PBL were again cultured *in vitro* for 3 days. SSF was added to these cultures at the beginning of this period. Fuc or Gal (50 m*M*) was then added to the cultures either at the beginning (0 hr) of the 3-day incubation, 2 hr after the addition of SSF, 24 hr after the addition of SSF, or 48 hr after the addition of SSF. Figure 4 demonstrates results from eight separate experiments. Addition of Fuc or Gal at 0 hr or 2 hr after the addition of SSF resulted in a statistically significant ($P < 0.05$) reversal of SSF-mediated suppression. However, addition of Fuc or Gal at 24 or 48 hr after addition of SSF did not result in any statistically significant reversal. These results suggest that SSF-mediated suppression of NK cytotoxicity becomes irreversible at some point between 2 and 24 hr after its addition to cell cultures.

6. Fucose-affinity chromatography of SSF. SSF-containing supernates were subjected to Fuc–agarose affinity chromatography as described above. The concentrated elutions from these columns were examined for their ability to modulate NK cytotoxicity. Data from a representative experiment are presented in Table 2, in terms of lytic units per 10^7 effector cells. SSF-containing supernate, before addition to the column, was able to suppress NK cytotoxicity by more than one-third. The eluate of the column after washing with 50 m*M* $NaPO_3$ (column buffer) and the final 3.5 *M* NaSCN elution caused only slight suppression of NK cytotoxicity, while the 1 *M* NaCl elution caused slight stimulation of NK cytotoxicity. The 4.5 *M* $MgCl_2$ elution, however, caused greater suppression of NK cytotoxicity than was seen with the initial crude (pre-column) SSF. This indicates that the $MgCl_2$ elution can enrich SSF. Two additional experiments produced similar results.

DISCUSSION

To examine the mechanisms involved in the interaction of an immunoregulatory lymphokine, SSF, and cell-surface molecules, the ability of monosaccharides to regulate SSF-mediated suppression was examined. Various investigators have demonstrated

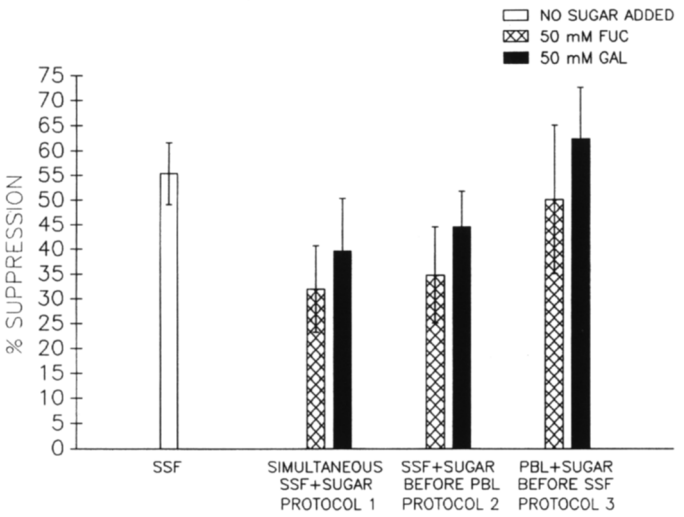

FIG. 3. Site of monosaccharide reversal of SSF-mediated suppression of NK cytotoxicity. Fresh nonadherent PBL were incubated in RPMI 1640 medium supplemented with 1% fetal calf serum for 3 days using one of 3 protocols: (1) SSF and 50 m*M* Fuc or Gal added simultaneously to PBL 24 hr after the start of the incubation period (protocol 1); (2) PBL cultured alone for 24 hr while SSF was incubated with either Fuc or Gal (50 m*M*); after 24 hr, the monosaccharide-SSF mixture was added to the PBL and cultured for an additional 48 hr (protocol 2); (3) PBL were cultured with 50 m*M* Fuc or Gal in the absence of SSF for 24 hr, after which SSF was added and the mixture cultured for an additional 48 hr (protocol 3). After 3 days of culture NK activity was determined against K562 targets at E:T ratios of 50:1. Percentage suppression was calculated as described under Materials and Methods, and mean values are represented. The vertical lines represent the standard errors of the means. The difference between % suppression for cells cultured in the absence of monosaccharides (SSF alone) and % suppression for cells cultured with SSF and 50 m*M* Fuc or Gal under protocols 1 or 2 is statistically significant at $P < 0.05$ while the difference between cells cultured with SSF alone and cells cultured with SSF and 50 m*M* Fuc or Gal under protocol 3 is not statistically significant.

that specific monosaccharides can reverse the actions of certain lymphokines. The activity of one of the most thoroughly studied examples, macrophage/monocyte migration inhibition factor, can be reversed by L-fucose and D-mannose (9). This appears to be a highly stereochemically specific interaction, as L-fucose but not D-fucose is capable of blocking MIF priming of rabbit alveolar macrophages (10). Other lymphokine actions reversed by monosaccharides include: inhibition of human fibroblast migration inhibitory factor activity by several monosaccharides (11); blocking of the activities of charge-reducing lymphokine(s) by α-L-fucose (12); blocking of a B cell-suppressive lymphokine by L-rhamnose (13); inhibition of macrophage fusion factor by α-D-mannose (14); and blocking of the actions of lymphocyte migration inhibitory factor by α-L-fucose (15).

The ability of monosaccharides to reverse SSF-mediated suppression of NK cytotoxicity has also been examined here. Six monosaccharides were initially screened for their effects on NK activity; four of these (Fuc, Gal, Glc, and Man) were shown to have no significant effect. Two other monosaccharides were observed to significantly suppress NK cytotoxicity directly, 6-MP (at 25 and 50 m*M* concentrations) and NANA (only at 50 m*M* concentration). The suppressive actions of 6-MP on NK cytotoxicity have previously been reported (16), and are thought to be due to inhibition of the

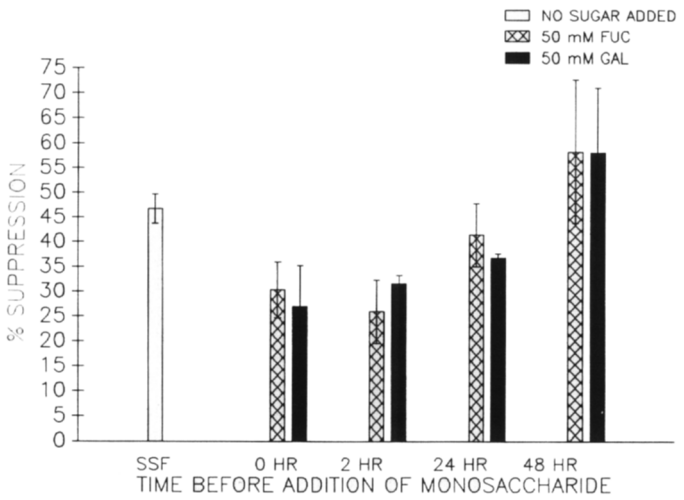

FIG. 4. Kinetics of monosaccharide reversal of SSF-mediated suppression of NK cytotoxicity. Fresh nonadherent PBL were incubated in RPMI 1640 medium supplemented with 1% fetal calf serum and 20% SSF. 50 m*M* Fuc or Gal was added to selected cultures at the start of the incubation period (0 hr), 2 hr after the start, 24 hr after the start, or 48 hr after the start. After 3 days total incubation, the NK activity of these cells was determined against K562 targets at E:T ratios of 50:1. Percentage suppression was calculated as described under Materials and Methods, and mean values are represented. The vertical lines represent the standard errors of the means. The difference between % suppression for cells cultured in the absence of monosaccharides (SSF alone) and % suppression for cells cultured with SSF and 50 m*M* Fuc or Gal added at 0 hr or 2 hr is statistically significant at $P < 0.05$ while the difference between cells cultured with SSF alone and cells cultured with SSF and 50 m*M* Fuc or Gal added at 24 hr or 48 hr is not statistically significant.

binding of NK effector cells to target cells. However, the suppressive actions of NANA on NK cytotoxicity have not been previously described. The effect of NANA on NK cytotoxicity is not due to altered pH, as the pH of the NANA solution was neutralized before addition to PBL. The mechanism by which NANA suppresses NK cytotoxicity is unknown.

TABLE 2

Effect of SSF Affinity Chromatography Fractions on NK Cytotoxicity[a]

Fraction	LU[b]	% Suppression
Control	235.2	—
SSF (prechromatography)	146.9	37.5
$NaPO_3$ wash (column buffer)	192.7	18.1
NaCl eluent	273.4	−16.2[c]
$MgCl_2$ eluent	107.2	54.4
SCN eluent	190.8	18.9

[a] PBL were cultured for 3 days with mock SSF (control), SSF, or fractions from affinity chromatography as 20% supplements. NK activity of these cells was then determined as described under Materials and Methods at effector:target cell ratios of 50:1.

[b] Lytic units per 10^7 effector cells, were calculated as described under Materials and Methods.

[c] Negative % suppression refers to LU greater than control values.

Fuc, Gal, Glc, and Man were next examined for their ability to block SSF-mediated suppression of NK cytotoxicity. Fuc and Gal were able to partially or completely reverse SSF-mediated suppression at various E:T ratios. Man and Glc were not able to significantly reverse SSF-mediated suppression. The stereochemistry of these sugars supports the hypothesis of a specific monosaccharide-mediated blockade of SSF's actions. Gal and Fuc (6-deoxy Gal) are very similar in stereochemical structure, having four chiral centers in common. In Glc, only three of these four chiral carbons (carbons 2, 3, and 5) possess the same configuration as found in Fuc and Gal, while Man has only two chiral centers with the same stereochemical structure as seen in Fuc and Gal (carbons 2 and 4). One hypothesis is that the reversal of SSF-mediated suppression is occurring via a stereospecific ligand-receptor interaction. Gal has the "best fit" in this "receptor," and is able to block SSF-mediated suppression at lower concentrations. Fuc has the same stereochemical arrangement as Gal, but has a dehydroxy carbon 6. As 25 m*M* Fuc is unable to significantly reverse SSF-mediated suppression at E:T ratios of 50:1, a hydroxylated carbon 6 may be important for optimal binding. Similar efficacies for L-Fuc and D-Fuc suggest that the stereochemistry of carbon 1 is unimportant for SSF-binding.

Glc, with three proper chiral centers, and Man, with only two proper chiral centers, do not bind as well, and are not able to significantly affect reversal of suppression even at the higher concentration tested. The higher concentration (50 m*M*) of Glc did appear to minimally reverse SSF-mediated suppression (i.e., 46.5% suppression without monosaccharide versus 42.6% suppression with 50 m*M* Glc at the E:T ratio of 50:1), although this reversal was not statistically significant. These results further support the hypothesis of a stereochemically specific interaction between SSF and a saccharide ligand.

The site of inhibition of SSF-mediated suppression, whether due to blockade of a cell-surface receptor for a monosaccharide-containing moiety or due to blocking of a monosaccharide-binding "receptor" within the SSF molecule, was also examined. Incubating SSF with Fuc or Gal before subsequent exposure to PBL produced a reversal of suppression statistically similar to that obtained by simultaneously adding SSF and either monosaccharide to PBL. Incubating PBL with either monosaccharide prior to the addition of SSF did not, however, produce a significant reversal of SSF-mediated suppression. Although preliminary, these results suggest that inhibition of SSF-mediated suppression is due to interactions between the SSF molecule and the monosaccharides, and not to interactions between a cell-surface molecule and the monosaccharides. This further suggests that SSF induces suppression by binding to monosaccharide-containing moieties on effector cell surfaces. Hence SSF may have lectin-like properties.

The highest concentration of Fuc or Gal tested (50 m*M*) was unable to completely reverse SSF-mediated suppression at every E:T ratio examined. However, these concentrations of 25 and 50 m*M* were chosen as being physiologically relevant, not supraphysiologic or pharmacologic. Higher, pharmacologic concentrations of Fuc or Gal could hypothetically completely block SSF-mediated suppression. However, at concentrations above 100 m*M*, these monosaccharides are capable of mediating immunosuppression of NK cytotoxicity in the absence of SSF (data not shown). Therefore, at these higher concentrations, it is difficult to separate monosaccharide-mediated suppression from reversal of SSF-mediated suppression due to the same monosaccharide. Further, as the "ligand" for SSF binding is presumably not a single mono-

saccharide, but, more likely an oligosaccharide, we did not expect any single monosaccharide at physiologic concentrations to completely reverse SSF-mediated suppression.

A number of other lymphokines have been shown to bind to effector cell surfaces via sugar-containing moieties. B151-TRF2, a factor derived from the B151K12 T cell hybridoma, can induce polyclonal differentiation of unprimed B cells (17). *N*-Acetyl-D-glucosamine (GlcNAc) can block this induction. In addition, B151-TRF2 can specifically bind and be eluted from GlcNAc-coated agarose beads, suggesting that this factor binds to GlcNAc-containing moieties on cell surfaces. A similar monosaccharide-mediated effect has been reported for the lymphokine responsible for activation of macrophage C3 receptors for phagocytosis (18). The actions of this lymphokine can be inhibited by L-Fuc. Pretreatment of macrophages with the enzyme fucosidase or with gorse lectin, a fucose-binding lectin, also inhibits the actions of this lymphokine, suggesting that it also binds to a Fuc-containing moiety found on the macrophage surface.

We examined the kinetics of monosaccharide blockade of SSF-mediated suppression. Fuc and Gal were able to partially inhibit SSF-mediated suppression of NK cytotoxicity after PBL had been exposed to SSF for 2 hr, but not after exposure for 24 hr. This indicates that the suppressive actions of SSF become irreversible with respect to the monosaccharides sometime during the first 24 hr of PBL exposure to SSF. Two possibilities are that after this time period, SSF-induced intracellular events can continue without SSF remaining bound to the cell surface, or that the binding of SSF to a cell surface molecule becomes irreversible and/or internalized.

There are few data available describing the kinetics of suppression in other lymphocyte systems, but what data are known suggest that the time course is highly variable in different systems. As with SSF, rapid suppression is seen in certain systems. For example, hybridomas developed from lactate dehydrogenase B (LDHB)-activated T suppressor cells produce a factor that suppresses proliferation of LDHB-activated T helper cells. Suppression is seen after 4 hr of culture with this factor (19). Rapid suppression also occurs in suppression of plasmacytoma MOPC-315 proliferation and IgA production by IgA-binding factors (20). This suppression is maximal within 8 to 12 hr. In other systems, however, suppression occurs only after more than 24 hr of exposure to the suppressive factor(s). Suppression of PHA-stimulated T cell transferrin receptor mRNA production by 1,25-dihydroxyvitamin D_3 (calcitriol) is not seen for up to 24–36 hr after PHA stimulation (21). Also, suppression of the mixed lymphocyte response (MLR) by a suppressive factor (MLR-TsF) is not maximal until 72 hr after MLR initiation (22). These differences indicate that a number of intracellular mechanisms and pathways can result in lymphocyte suppression. The suppression mediated by SSF apparently involves an early-acting mechanism and pathway.

In view of the data suggesting monosaccharide binding by SSF, concentration of SSF by fucose affinity chromatography was attempted. One fraction recovered from the fucose-linked agarose bead affinity column, eluted by a high salt concentration (4.5 *M* $MgCl_2$), showed greater suppression of NK cytotoxicity than did the crude SSF applied to the column. This suggests that this eluate contained concentrated SSF, and strengthens the hypothesis that SSF binds specific monosaccharides.

Many reports suggest that monosaccharides may be involved in regulation of the immune system. A number of studies have examined the role of Man in immunoregulation. Although we found Man to have no significant affect on NK activity, Man has been reported to suppress antigen-specific proliferative assays (23). Tosato and co-

workers (24) demonstrated that Man can reverse suppression of pokeweed mitogen-stimulated immunoglobulin production mediated by infectious mononucleosis-activated suppressor T cells. A Man-containing disaccharide has also been found in human pregnancy urine that is capable of inhibiting proliferative responses of human T lymphocytes (25).

Monosaccharide-mediated blockade of lymphokine activities may also play a role in immunoregulation, particularly in certain clinical conditions. Antigen-specific desensitization of cellular immunity, achieved by administration of large doses of antigen, can be blocked by Fuc (26). Fuc can also suppress the contact allergic response to dinitrofluorobenzene in BALB/c mice (27). These experiments suggest that inhibition of lymphokine actions by monosaccharides can indeed play a role in regulating the immune response under normal conditions as well as in certain disease states. It may become possible to regulate specific lymphokines *in vivo* by the administration of targeted monosaccharides.

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