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Plasma Inhibition of Lymphocyte Proliferation in Nephrotic Syndrome: Correlation with Hyperlipidemia

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Plasma-mediated inhibition of normal lymphoproliferation is an unexplained immunologic abnormality frequently observed in nephrotic syndrome. Since hyperlipidemia, also common in nephrotic syndrome, has been linked with *in vitro* and *in vivo* immunodeficiency in other diseases, we have quantitated plasma-mediated inhibition of lymphoproliferation and related it to the degree of hyperlipidemia in 19 patients with nephrotic syndrome. Fifteen patients were hyperlipidemic; the plasma of 9 of these 15 caused >60% inhibition of antigen-specific proliferative responses of normal lymphocytes. None of the four normolipidemic plasmas, nor a hyperlipidemic plasma depleted of lipoproteins by ultracentrifugation, was inhibitory. A highly significant correlation between the degree of inhibition and the plasma triglyceride levels in patients with nephrotic syndrome was observed ($P < 0.001$). The results suggest that elevated plasma lipids may be the cause of the plasma-mediated inhibition of lymphoproliferation in nephrotic syndrome.

KEY WORDS: Lymphocyte proliferation; plasma factors; hyperlipidemia; nephrotic syndrome.

INTRODUCTION

Nephrotic syndrome, whose pathogenesis has not been defined, is characterized by proteinuria, hypoproteinemia, edema, and hyperlipidemia. Several defects in immune function have been observed in patients with nephrotic syndrome (1–5). One of these is an inhibitory effect of their plasma on normal lymphoproliferative responses to mitogenic stimulation in culture (6–8). We have investigated the possibility that this inhibition of lymphoproliferation in nephrotic syndrome might be linked to the associated hyperlipidemia, since hyperlipidemic plasma in several other clinical states has been shown to have a similar inhibitory effect (9, 10).

MATERIALS AND METHODS

Nineteen patients (1 to 23 years old) with nephrotic syndrome of varying degrees of severity were studied. The causes of nephrotic syndrome in the patients studied were as follows: minimal change, 6; focal glomerular sclerosis, 4; membranoproliferative glomerulonephritis type II, 3; membranous nephropathy, 2; congenital nephrosis, 1; Henoch–Schonlein purpura, 1; and acute glomerulonephritis, 2.

After informed consent was obtained, blood samples were drawn in preservative-free heparin and the plasma was separated by centrifugation. Triglyceride and cholesterol concentrations were determined using enzymatic assays. (11, 12).

Peripheral blood mononuclear cells (PBM) for the lymphoproliferation studies were obtained from heparinized blood samples of healthy donors by Ficoll–Hypaque density gradient centrifugation (13). The proliferative responses of these normal PBM cultured in 7.5% (v/v) patient or control normal plasma were determined by the incorporation of [^{3}H]thymidine as previously described (14). The stimulants of lymphoproliferation, used at optimal concentrations, were the nonspecific mitogens phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM), and the specific soluble antigens candida antigen and streptokinase–streptodornase (SKSD). The net [^{3}H]thymidine uptake was determined by subtracting the [^{3}H]thymidine uptake by unstimulated triplicate

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$$\frac{\text{(net cpm in control plasma)} - \text{(net cpm in nephrotic plasma)}}{\text{(net cpm in control plasma)}} \times 100.$$

cultures from that by triplicate cultures incubated in the presence of a mitogen or antigen.

The percentage change in proliferation caused by nephrotic plasma was calculated, see equation above. We previously determined the geometric mean response of normal PBM cultured in allogeneic normal plasma to be 95 ± 53% ($\bar{X}$ ± 2 SD, N = 12) of their response when cultured in autologous plasma (15). Therefore, we defined plasma of a patient with nephrotic syndrome to be inhibitory if it reduced the proliferative response of normal PBM to less than 42% of that observed in autologous normal plasma. The exact quantitative relationship between plasma-mediated inhibition and plasma lipid levels was determined by linear regression analysis.

Plasma lipoproteins were separated from one nephrotic plasma to determine directly whether the plasma inhibitory activity was associated with the lipoprotein fraction. Briefly, the plasma was adjusted to a density of 1.23 with solid potassium bromide and then ultracentrifuged, as described (16), yielding two fractions. These, the total lipoprotein fraction (which contained >95% of the total plasma triglyceride and cholesterol) and the lipoprotein-depleted plasma fraction, as well as an aliquot of the whole plasma, were exhaustively dialyzed against phosphate-buffered saline (pH = 7.4). After dialysis, the fractions were reconstituted to the original plasma volume to allow subsequent direct comparison of their relative inhibitory effects on normal lymphoproliferation.

RESULTS

Plasma triglyceride concentrations of the 19 patients with nephrotic syndrome ranged from 68 to 630 mg/dl (313 ± 42 mg/dl, $\bar{X}$ ± SE). Fifteen of the 19 patients had hypertriglyceridemia, defined as a plasma triglyceride concentration more than 2 SD above the nonfasting normal mean, matched for age, sex, and race (17). This upper limit of normal, which has been found to be not significantly affected by fasting (17, 18), ranged between 94 and 174 mg/dl for these 19 patients.

Plasma cholesterol concentration (measured in 18 patients) ranged from 111 to 665 mg/dl (314 ± 42 mg/dl). Fourteen of the 18 patients were hypercholesterolemic by similar criteria, with the age-, sex-, and race-related nonfasting upper limit of normal ranging between 193 and 210 mg/dl (17).

When individual patients' plasma triglyceride and cholesterol levels were compared, a highly significant linear correlation between the two lipid levels was found (r = 0.89, $P < 0.001$). We have used the plasma triglyceride level to report the quantitative correlation between the inhibition of lymphoproliferation and the degree of hyperlipidemia. However, as would be expected from the high correlation between plasma triglyceride and cholesterol levels in this group of patients, results similar to those presented below were obtained using the plasma cholesterol concentration to quantitate hyperlipidemia (not shown).

At a concentration in culture of 7.5% (v/v), plasma of 7 and 9 of the 15 patients with elevated triglyceride concentrations inhibited proliferative responses of normal PBM to the specific antigens, candida and SKSD, respectively. The degree of inhibition varied but was greater than 90% in the case of seven of the plasmas (Fig. 1). Inhibition of the response to nonspecific mitogens was less fre-

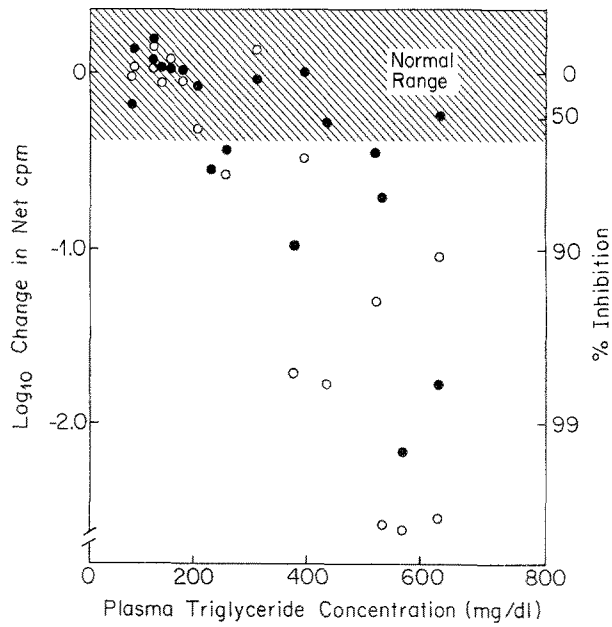

Fig. 1. Effect of nephrotic syndrome patient plasma on normal lymphoproliferation. Each point represents the change in the proliferative response of normal PBM to the specific antigen Candida (●) or SKSD (○), when cultured in 7.5% (v/v) patient vs control normal plasma. Abcissa: patient plasma triglyceride concentration.

Table I. Effect of Varying Concentrations of Normal Plasma and Nephrotic Plasma on Normal Lymphoproliferation *in Vitro*

% Plasma (v/v) in culture		Net [^{3}H]thymidine uptake[a]	
Normal	Nephrotic[b]	cpm	% of control
7.5 (control)	0	7954[c]	(100)
1.5	0	8747	109
0	7.5	213	2
1.5	6.0	132	1
3.75	3.75	476	5
6.0	1.5	547	6

[a]Stimulant, SKSD.
[b]Plasma triglyceride concentration, 562 mg/dl.
[c]Mean of triplicate cultures; SE was <10%.

quently observed; one to three of seven hyperlipidemic plasmas tested were inhibitory, depending on the mitogen being tested. Significantly, plasma of the four normolipidemic nephrotic syndrome patients did not inhibit lymphoproliferative responses to any stimulant (mitogen or antigen). The inhibitory effect of patient plasma was not due to direct cytotoxicity, since, compared with culture in control plasma, culture for 6 days in patient plasma did not reduce PBM number or cell viability of unstimulated cultures, as assessed by trypan blue dye exclusion (not shown).

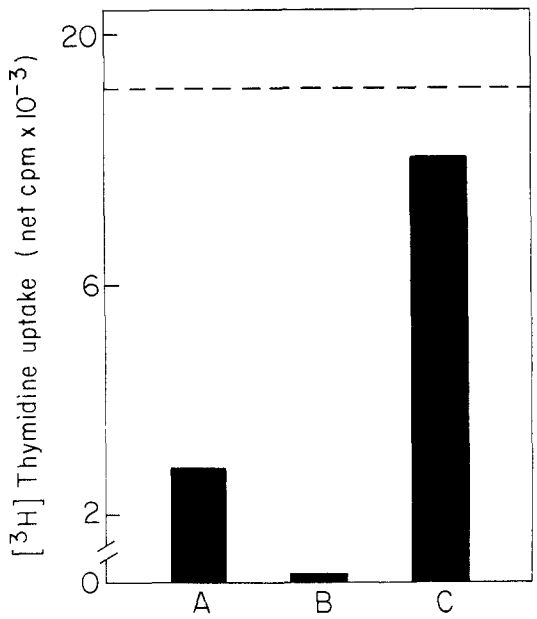

Fig. 2. Effect of lipoprotein depletion on the inhibitory activity of nephrotic plasma. The proliferative response of normal PBM stimulated with SKSD was measured. All cultures contained 1.5% (v/v) autologous (normal) plasma. In addition, 6% (v/v) of either dialyzed allogeneic normal plasma (dashed line; control), dialyzed nephrotic plasma (A), lipoprotein-rich nephrotic plasma (B), or lipoprotein-depleted nephrotic plasma (C) fractions was added. Triglyceride concentrations in A–C were 411, 365, and 20 mg/dl, respectively.

Experiments were performed to determine whether the observed depression of proliferative responses of normal PBM cultured in patient plasma represented a plasma inhibitory effect or a lack, in patient plasma, of a normally present factor essential for lymphoproliferation. As little as 1.5% (v/v) normal plasma supported proliferation of normal PBM, but the addition of this amount of normal plasma did not overcome the inhibition of proliferation caused by 6% (v/v) patient plasma (Table I). These data indicate the presence of inhibitory activity rather than the lack of an essential plasma factor in plasma of patients with nephrotic syndrome.

Ultracentrifugation of an inhibitory patient plasma was performed to partially characterize the inhibitory activity by comparison of the resultant lipoprotein-rich and lipoprotein-depleted plasma fractions for their effects on normal lymphoproliferation. As in the previous experiment, all cultures contained 1.5% (v/v) normal plasma to ensure that any inhibitory effects were not caused by the lack of an essential factor in the nephrotic plasma fractions. Under these conditions, 6% (v/v) unseparated nephrotic plasma and the isolated lipoprotein-rich fraction each markedly inhibited the normal lymphoproliferative responses to SKSD (Fig. 2). In contrast, as also shown in Fig. 2, lipoprotein depletion almost completely abrogated the inhibitory effect of the patient plasma. These results strongly support the suggestion that the inhibitory activity in nephrotic plasma is associated with the plasma lipoproteins.

To ascertain the quantitative relationship in nephrotic syndrome between plasma triglyceride concentration and plasma-mediated inhibition of normal human lymphoproliferation, a linear regression analysis was performed (Table II). A highly significant correlation between plasma-mediated inhibition of antigen-specific lymphoproliferative responses and triglyceride concentration was found. Inhibition of the responses to two of the three mitogens tested was similarly related to triglyceride concentration. However, the magnitude of inhibition (slope) of the antigen-specific lymphoproliferative responses was three- to ninefold greater than the magnitude of inhibition of the mitogen-induced responses (Table II). This indicates that antigen-specific responses are more sensitive to inhibition by plasma from patients with nephrotic syndrome.

Table II. Correlation Between the Plasma Triglyceride Concentration and the Degree of Plasma-Mediated Inhibition of Normal Lymphoproliferation in Nephrotic Syndrome

Stimulant of lymphoproliferation	No. of plasmas tested	Slope[a]	r^b	P^c
Specific antigens				
Candida antigen	19	0.0023	0.697	<0.001
SKSD	18	0.0036	0.770	<0.001
Nonspecific mitogens				
Con A	12	0.0008	0.835	<0.001
PHA	10	0.0008	0.739	<0.01
PWM	12	0.0004	0.470	>0.05

[a]Slope was determined by linear regression analysis of the $\log_{10}$-normalized changes in net [^{3}H]thymidine uptake of normal PBM cultured in the 19 patient plasmas (vs control normal plasma) compared with the corresponding patient plasma triglyceride concentrations. The equation for the slope of the resulting line is Δ ($\log_{10}$ net cpm in control plasma − $\log_{10}$ net cpm in patient plasma)/Δ patient plasma triglyceride concentration.
[b]Correlation coefficient.
[c]Significance level, determined by Student's t test, two tailed.

However, by increasing to 15% (v/v) the concentration of a patient plasma which inhibited only antigen-specific responses when used at a concentration of 7.5% (v/v), a >90% decrease in normal lymphoproliferative responses to the three mitogens, compared with their response in 15% normal plasma, was also observed. The uniform inhibition of proliferative responses to both mitogens and antigens by the higher concentration of patient plasma suggests that the selectively greater inhibition of antigen-specific lymphoproliferative responses at the lower (7.5%) plasma concentration reflects a quantitative and not a qualitative difference.

DISCUSSION

The possibility that patients with nephrotic syndrome may be clinically immunodeficient was initially suggested by their increased susceptibility to bacterial infections (19). Abnormalities of humoral and cellular immunity which have been demonstrated include decreased bactericidal capacity of polymorphonuclear neutrophils and decreased serum opsonic activity (1), low serum levels of IgG (4), and depressed delayed hypersensitivity skin test reactions to SKSD (5).

Abnormal plasma factors (possibly causing glomerular injury resulting in increased permeability) were first suspected to be associated with nephrotic syndrome when recurrence of this syndrome followed renal transplantation in three patients (20). Shalhoub postulated that an abnormal clone of T lymphocytes was producing this factor (21). Subsequently, it was found that plasma from patients with minimal-change nephrotic syndrome inhibited normal lymphoproliferative responses *in vitro* (6–8). Similar observations have been noted in patients with nephrosis as a secondary manifestation of other disease processes (22), and plasma from rats with experimentally induced nephrosis has also been found to have similar immunosuppressive properties (23).

As the cause of this plasma inhibitory activity in nephrotic syndrome had not been defined, we undertook the present study to determine if the plasma-mediated inhibition of lymphoproliferation in nephrotic syndrome might be related to the hyperlipidemia also observed in this syndrome. Several previous observations suggested this possibility. Plasma inhibitory activity has previously been associated with hyperlipidemia in patients with several other clinical conditions, including, for example, familial hyperlipidemia (9) and one form of childhood histiocytosis (24). Both low-density and very low-density lipoproteins have immunoregulatory properties (16, 25) and may be elevated in plasma of patients with nephrotic syndrome. These observations led to the recent suggestion (26) that lipoproteins may account for the plasma inhibitory activity in this syndrome, a possibility supported by the isolation from nephrotic plasma of a low-density lipoprotein-containing fraction having such inhibitory activity (27).

The present findings demonstrating a direct correlation between the level of hyperlipidemia and the degree of plasma-mediated immunosuppression in nephrotic syndrome provide further evidence that circulating lipids may be responsible for this immunologic abnormality. Supporting this conclusion are also the facts that (i) none of the plasma samples from patients with nephrotic syndrome who were normolipidemic at the time of study had any inhibitory effect on normal lymphoproliferation, and (ii) separation of the total lipoprotein fraction from an inhibitory nephrotic plasma almost completely abrogated the plasma inhibitory activity.

The number of patients studied was too small to draw conclusions about a relationship between the etiology of nephrotic syndrome and the presence of either hyperlipidemia or plasma-mediated immunosuppression. However, our findings agree with previously published reports that the inhibitory effects

on lymphoproliferation are not limited only to the plasma of patients with nephrotic syndrome of the minimal-change type, but may be seen in nephrotic syndrome of various etiologies (20, 28).

The *in vivo* significance of the hyperlipidemia-associated, plasma-mediated inhibition of lymphoproliferation in nephrotic syndrome remains to be established. In this context, it is of interest that an apparently beneficial effect of plasma-mediated immunosuppression in chronic renal disease has recently been reported by Veitch *et al.* (29). These authors prospectively quantitated plasma-mediated inhibition of lymphoproliferation in patients with chronic renal failure who subsequently received renal allografts and found that the presence of plasma inhibitory activity was associated with a lower incidence of early posttransplant graft rejection. Therefore, studies to identify directly the factor(s) responsible for plasma inhibitory activity should be continued.

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