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Role of a SER immune suppressor in immune surveillance

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SUMMARY

A potent immunosuppressor factor, known as SER (suppressive E-receptor factor) has been identified in the body fluids of cancer patients. SER has been proven to be immunochemically analogous to the fetal form of haptoglobin. In this paper, we examine the role of SER immune suppressor in the immune surveillance mechanism of the host, using an affinity-purified SER. As shown in this study, SER, at μg/ml concentrations, inhibits the T-cell proliferation induced with either monoclonal or polyclonal T-cell activators *in vitro* in human, and also inhibits the primary antibody response to T-dependent antigens *in vivo* in mice. Likewise, SER also inhibits the immunoglobulin synthesis of human B lymphocytes induced by a B-cell mitogen, pokeweed mitogen, in the presence of a tumour promoter, phorbol myristate acetate (PMA). In contrast to the T-dependent antibody response *in vivo* in mice or T-dependent mitogen response *in vitro* in human, SER does not interfere with the T-independent antibody responses to DNP–Ficoll or TNP–LPS in mice. SER also interferes with the natural killer cell function of human peripheral blood mononuclear cells. Although SER inhibits the phagocytic functions of human peripheral neutrophils, it requires at least 10–20 times the concentration of SER present in normal human plasma. Since this concentration of SER is attainable in the sera of solid tumour-bearing patients, highly elevated levels of SER could predispose the patients to microbial infections as well. This study demonstrates that purified SER manifests multi-faceted down-regulatory effects on the defence mechanism of hosts, thereby it could compromise the patients' cell-mediated immunity *in vivo*.

INTRODUCTION

Cellular immune responses can be modulated by factors present in normal plasma (Kamrin, 1959). Patients with metastatic cancer often elaborate such factors, compromising immune surveillance (Glasgow *et al.*, 1974; Badger *et al.*, 1977; Hess, Gall & Dawson, 1980). In addition to the antigen-specific modulators, which interact with the major histocompatibility complex, numerous antigen-non-specific modulators have been identified. These include the normal immunosuppressive protein (NIP) (Glaser & Nelken, 1972; Nelken, 1973; Nelken, Ovadia & Hanna, 1979; Glaser, Tring & Herberman, 1975; Goren & Nelken, 1981), the immunoregulatory alpha-globulin (IRA) (Cooperband *et al.*, 1968; Menzoian *et al.*, 1974), components of acute-phase reactants, e.g. alpha-1-acid glycoprotein (Bennett & Schmid, 1980), haptoglobin (Baseler & Burrell, 1983), alpha-1-anti-trypsin (Breit *et al.*, 1985) and certain complement breakdown products (Weigle *et al.*, 1982).

Our laboratory isolated a potent immunosuppressive factor, suppressive E-receptor factor (SER), from malignant ascites fluids from patients with ovarian cancer (Oh & Moolten, 1981a). This factor, which also inhibited E-rosetting, was immunochemically identical to the fetal form of haptoglobin (Oh *et al.*, 1987a). SER had many of the properties of IRA (Menzoian *et al.*, 1974), and functioned as an anti-proliferative factor on T-dependent responses *in vitro* (Oh & Moolten, 1981a). It did not alter the spontaneous proliferation of most tumour or fibroblast cells *in vitro* (Oh *et al.*, 1987b). The detailed biochemical action of SER on T-cell activation is still not known, but it has been shown to selectively inhibit the DNA-polymerase-alpha activity of human lymphoblasts (Oh & Lapenson, 1985), presumably via the T11 alternate activation site of T lymphocytes (Oh *et al.*,

Abbreviations: ABTS, 2,2′-azino-bis-(3-ethyl-benzo thiazoline)-6-sulphonic acid; CRP, C-reactive protein; DNP–Ficoll, dinitrophenyl-lysine–Ficoll; IRA, immunoregulatory alpha-globulin; NIP, normal immunosuppressive protein; PBS, phosphate-buffered saline (0·01 M sodium phosphate, pH 7·4 + 0·15 M NaCl); PHA, phytohaemagglutinin; PMA, phorbol-12-myristate-13-acetate; PMN, polymorphonuclear leucocytes; PWM, pokeweed mitogen; SER, suppressive E-receptor factor; SRBC, sheep red blood cells; TBS, Tris-buffered saline (0·05 M Tris-HCl, pH 7·0, + 0·15 M NaCl); TNP–LPS, trinitrophenylated–lipopolysaccharides.

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1984). In this study, we examined the role of affinity-purified SER in modulating the immune surveillance mechanism of the host *in vivo* and *in vitro*.

MATERIALS AND METHODS

Purification of SER from malignant effusions or plasma

Procedures developed in our laboratory (Oh & Moolten, 1981a) and those of Rademacher & Steele (Rademacher & Steele, 1987) were used. Prior to purification, the content of haptoglobin in the fluid was determined using an enzyme-linked immunoassay (EIA) with a purified haptoglobin standard (type 2-2) (Sigma Chemicals, St Louis, MO). Briefly, malignant effusion or plasma derived from cancer patients was partially delipidated as described previously (Burstein, Scholnick & Marfin, 1970) and the resulting precipitate was removed by centrifugation at 10,000 *g* for 10 min at 4°. The supernatant fraction was dialysed against Tris-buffered saline (TBS) (0·05 M Tris-HCl, pH 7·0 + 0·15 M NaCl) overnight at 4°. The delipidated and dialysed fluid was adsorbed onto the chicken cyanomethemoglobin affinity media at room temperature (22°). Affinity media was produced with Sepharose CL-4B by conjugation with chicken cyanomethemoglobin, as described by Rademacher & Steele (1987) and the column was equilibrated with the TBS. Haptoglobin, bound to the affinity media, was eluted with freshly prepared 8 M urea (Sigma Chemicals) dissolved in TBS. Fractions eluted in the ascending portion of the column that did not contain urea were pooled and dialysed against TBS with 2·5 mM $CaCl_2$ and 0·1 mM $CoCl_2$ for subsequent purification. The urea-free fraction obtained from this affinity media was further purified using concanavalin A (Con A)–Sepharose media (Pharmacia Fine Chemicals, Uppsala, Sweden). Fractions that did not bind the Con A column (fraction A) and the bound fractions eluted with 0·1 M alpha-D-methyl-mannoside (fraction B) were collected separately. Fraction B was dialysed against phosphate-buffered saline (PBS) overnight at 4° in order to remove the excess sugar. Both of these fractions were assayed on phytohaemagglutinin (PHA)-induced or anti-T3-induced mitogen assay of human peripheral blood T lymphocytes. Fraction A retained most of the immunosuppressive activity and was used as SER. Purity of the SER prepared above was examined by SDS–PAGE (12% gel), as described by Laemmli (1970), following vigorous reduction with 5% 2-mercaptoethanol in 1% SDS at 100° for 10 min.

Mitogen response assay in vitro

Detailed procedures for inhibition on mitogen-induced human peripheral blood T-cell activation have been already described in our previous publications (Oh & Moolten, 1981a, b). The ability of SER to inhibit the mitogen response of human peripheral blood mononuclear cells to pokeweed mitogen (PWM) or Con A was examined using varying concentrations of mitogens with a constant dose of SER. The dose of SER used to examine these mitogens was known to cause at least 50% inhibition of PHA- or anti-T3-induced mitogenesis.

Assay for the natural killer cell activity

Human natural killer cell (NK) activity was determined as originally described by Jondal, Spina & Targen (1978) using ^{51}Cr-labelled K562 cells as target cells and the human peripheral blood mononuclear cells as effector cells in a 4-hr assay. The percentage inhibition of NK activity was calculated as follows:

$$\frac{\text{maximum releasable c.p.m.} - \text{c.p.m. from the test culture}}{\text{maximum releasable c.p.m.} - \text{c.p.m. from spontaneous release}} \times 100.$$

Immunoglobin synthetic functions of B lymphocytes induced with PWM in the presence and absence of phorbol myristate acetate (PMA)

Peripheral blood mononuclear cells were stimulated with PWM (Gibco, Grand Island, NY) at 1·0 μg/ml of lymphocyte suspension (1×10^6 cells/ml) and cultured in RPMI-1640 (Gibco) medium containing 15% fetal calf serum, 100 μg/ml of gentamycin (Sigma Chemicals), 2 mM L-glutamine (Gibco), 100 U/ml of penicillin and 100 μg/ml of streptomycin (Gibco) for 7 days at 37° with 5% CO_2.

Some cultures also received 10 ng/ml of phorbol-12-myristate-13-acetate (PMA) and various doses of SER. At the end of the 7-day culture, cells were harvested and the culture supernatants were saved for determination of IgG and IgM synthesized. These immunoglobulins were measured by a double-determinant sandwich-type enzyme-linked immunoassay (EIA), similar to that of Ceuppens & Goodwin (1982), with the following modifications. The modifications include the use of sandwich-type double determinant with goat and rabbit antisera to human immunoglobulins and use of biotinylated goat anti-rabbit IgG as the detector antibody. The solid-phase bound biotin was monitored with avidin-conjugated glucose oxidase enzyme. The substrates for this reaction included horseradish peroxidase, glucose and 2,2′-azino-bis-(3-ethyl benzothiazoline)-6-sulphonic acid (ABTS), as described by Morgan & McIntyre (1983). Rabbit antiserum to human IgG (Behringwerke-Calbiochem, La Jolla, CA) was used at 1:5000 dilution and the rabbit antiserum to human IgM (Behringwerke-Calbiochem) was used at 1:1000 dilution.

Purified human IgG (Miles Lab., Indianapolis, IN) or IgM (Jackson ImmunoResearch Lab., West Grove, PA) was used as antigen standard. The colour intensity that developed with this chromogenic substance was measured with a Multiscan Plus II spectrophotometer (Flow Lab., McLean, VA) at 405 nm and compared with IgG or IgM standard curve.

A similar culture was also set up in microtitre plates and pulsed with [^{3}H] thymidine ([^{3}H]TdR; 0·5 μCi/well) for 6 hr on Day 3 and harvested onto a glass-fibre filter paper. The cellular proliferation determined by [^{3}H]TdR incorporation into DNA was measured in a similar manner as described above for the mitogen assay.

Phagocytosis assay

The red blood cell and leucocyte-containing pellets were obtained from the Ficoll–Hypaque density gradient separation of human peripheral blood and the polymorphonuclear leucocytes (PMNs) were isolated from these pellets as described by Boyum (1974). Phagocytosis was assessed by measuring the [^{3}H]uridine ([^{3}H]UdR) incorporated into added yeast cells. Uridine does not penetrate the PMN well, thus ingested yeasts cannot accumulate it, while yeasts which have not been ingested can (Yamamura, Boler & Valdimarsson, 1977). Prior to use, the yeast cells of *Candida albicans* were harvested, washed, and suspended in RPMI-1640 medium containing 5% human AB serum at a cell density of 5×10^6 cells/ml. PMNs prepared above

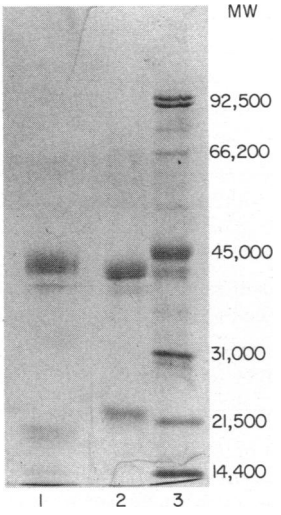

Figure 1. SDS–PAGE profile of SER and normal adult haptoglobins. Fraction A, which did not bind to Con A–Sepharose column was used as SER haptoglobin on lane 1, and haptoglobin standard (type 2-2) was run on lane 2 in 12% acrylamide gel. Molecular weight standards were run on lanes 3. Molecular weight standards included phosphorylase b (92,500 MW), bovine serum albumin (66,200 MW), ovalbumin (45,000 MW), carbonic anhydrase (31,000 MW), soy bean trypsin inhibitor (21,500) and lysozyme (14,400).

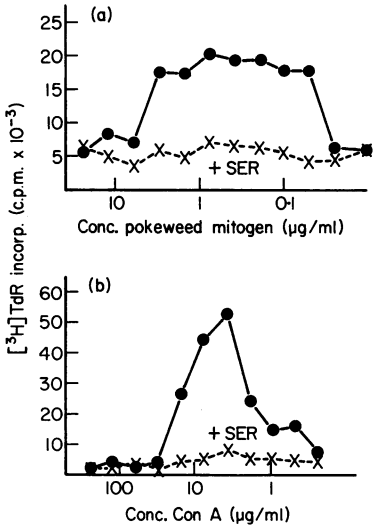

Figure 2. (a) Effect of an affinity-purified SER haptoglobin on pokeweed mitogen response of human peripheral blood mononuclear cells (PBMC). Purified human PBMC in culture were stimulated with pokeweed mitogen at the doses indicated and purified SER (fraction A, 2 μg/ml) was added to this culture. Effect of SER haptoglobin on mitogen response was measured by the [³H]TdR incorporated to DNA on Day 3 (pulsed with 0·5 μCi [³H]TdR/0·25 × 10^6 cells/well for the final 6 hr in 72-hr culture). [³H]TdR incorporated to DNA was measured by harvesting the cultures into glass-fibre filter discs and counting the filter discs in a Beckman liquid scintillation counter. (b) Effect of an affinity-purified SER-haptoglobin on human PBMC response to Con A. Purified SER haptoglobin (fraction A, 2 μg/ml) was added to the PBMC cultures stimulated with varying doses of Con A as indicated. Effect of SER haptoglobin on the proliferative response of PBMC to Con A was measured by [³H]TdR incoprorated to DNA on Day 3. [³H]TdR incorporated to DNA was measured in a similar manner as in Fig. 2a.

(2×10^5 cells/100 μl) were pre-incubated with various concentrations of SER, normal adult haptoglobin, alpha-l-acid glycoportein or *C*-reactive protein standard serum (added in 50 μl volume) for 20 min at 37° prior to exposure to *C. albicans*. One-hundred microlitres of *C. albicans* (5×10^6 cells/ml) were added to 150 μl of a PMN suspension containing 2×10^5 PMN cells and the test substance. An additional 50 μl volume of medium containing 5% human AB serum was added to make a total volume of 300 μl per assay. Cultures were incubated for another 60 min at 37° for phagocytosis to occur. Thereafter, 1×10^6 c.p.m. of [³H]UdR (DuPont-New England Nuclear, Boston, MA) were added to each well and incubated for 40 min at 37°. Next, the cells were harvested onto filter discs using the Titertek cell harvester (Flow Lab.). The [³H]UdR incorporated into RNA by *C. albicans* was determined by counting the radioactivity of the filter discs with 5 ml of Scintiverse (Fishers Scientific, Medford, MA) in a Beckman liquid scintillation counter Model LS-200 (Beckman Inst., Somerset, NJ). Control cultures were set up with *C. albicans* alone in the absence of PMNs and the [³H] UdR incorporated to RNA in this culture was considered as the maximum incorporation of [³H]UdR. Phagocytosis index was calculated as follows:

$$\text{phagocytosis index} = \frac{\text{max. c.p.m. with } C.\ albicans - \text{c.p.m. with test culture}}{\text{max. c.p.m. obtained with } C.\ albicans \text{ alone}} \times 100.$$

The values for the test cultures were normalized to be expressed as a percentage of control phagocytosis.

Antibody response to T-dependent and T-independent antigens in mice

Adult mice of BDF_1 strain were primed with 100 μg of purified SER in saline i.v. on Day 0 and subsequently immunized with either the T-dependent antigen or T-independent antigens. Sheep red blood cells were used as T-dependent antigen (0·5 ml of 10% suspension per mouse) and dinitrophenyl-lysine-Ficoll (DNP–Ficoll) or trinitrophenylated–lipopolysaccharide (TNP–LPS) utilized as the T-independent antigen (10 μg per mouse by i.p.). DNP–Ficoll was prepared according to the procedure described by McMaster, Bachvaroff & Rapaport (1975) and was found to contain an average of 38·5 residues of DNP conjugated per Ficoll moiety by Spectrophotometric analysis. TNP–LPS was prepared according to the procedure described by Jacobs & Morrison (1975). Both DNP–Ficoll and TNP–LPS were gifts from Dr E. Levy (Department of Microbiology, Boston University School of Medicine). Mice were killed on Day 4 following the immunization, and a single-cell suspension was prepared from their spleens. The direct plaque-forming cell response was determined by the modified Jerne haemolytic plaque assay (Jerne, Nordin & Henry, 1963) using the trinitrophenylated–sheep red blood cells (TNP–SRBC) as target cells in the presence of guinea-pig complement (Gibco). TNP–SRBC were prepared by the procedure described by Rittenberg & Pratt (1969).

RESULTS AND DISCUSSION

The purity of the affinity-purified SER is shown in Figure 1. The inhibitory effect of this purified SER on poyclonal human T-cell activators such as PHA or anti-T3 antibody was confirmed as previously reported (Oh *et al.*, 1987b).

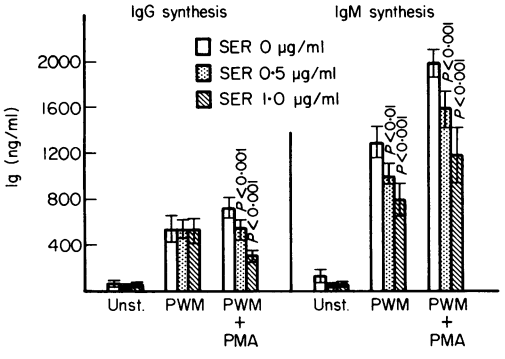

Figure 3. Effect of SER haptoglobin on IgG and IgM synthesis of human PBMC driven by PWM in the presence and absence of PMA. Human PBMC were stimulated with pokeweed mitogen (1 μg/ml) in the presence and absence of PMA (10 μg/ml) for 7 days in RPMI-1640 medium with 15% fetal calf serum, 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin and 100 μg/ml of gentamycin at 37° in a 5% CO_2 containing incubator. Culture supernatants were harvested at the end of this culture period and the IgG and IgM contents of the culture media were determined by a double-determinant sandwich-type enzyme-linked immunosorbent assay. Goat and rabbit antisera to immunoglobulins G and M were used as capture antibodies and biotynylated goat anti-rabbit IgG was used as detector antibody. The biotin bound to the immune complex was assayed with glucose oxidase, conjugated to avidin. ABTS, glucose and horseradish peroxidase were used as substrates for glucose oxidase enzyme.

Normal human lymphocyte responses to T-dependent mitogens, PWM or Con A were inhibited by SER, irrespective of the mitogen doses tested (Fig. 2a, b). Since the primary effect of SER on lymphocytes is its anti-proliferative effect, we examined whether SER also affects the synthesis of immunoglobulins in human peripheral blood lymphocytes induced by PWM, a T-dependent B-cell mitogen, in the presence and absence of PMA.

Effect of SER on immunoglobulin synthetic functions of B lymphocytes driven by PWM with and without PMA

As shown in Fig. 3, IgG synthesis driven by a B-cell mitogen, PWM, was relatively resistant to the inhibitory effect of SER. In contrast, the IgG synthesis promoted by PMA was significantly inhibited by the presence of SER ($P<0·001$ at 0·5 μg/ml and 1·0 μg/ml doses of SER). SER was also an effective inhibitor for IgM synthesis driven by PWM in the presence or absence of PMA ($P<0·01$ at 0·5 μg/ml SER and $P<0·001$ at 1·0 μg/ml of SER). This inhibitory effect of SER on immunoglobulin (IgG and IgM) synthesis in the presence of PMA was in parallel to its anti-proliferative response to PWM examined on Day-3 cultures (data not shown). As the PWM-driven immunoglobulin synthesis of B lymphocytes requires T-cell co-operation (Waldman & Broder, 1983), SER may also hinder the T-cell co-operation necessary for immunoglobulin synthesis in human B lymphocytes. Therefore, we examined the effect of SER on the primary antibody responses to T-dependent and T-independent antigens *in vivo* using BDF_1 mice.

Table 1. Antibody response to T-dependent and T-independent antigens *in vivo*

Group	Conditions	PFC† response
A	Control with saline + SRBC	23,900 ± 9600
	SRBC + mg human IgG	35,200 ± 13,200
	SRBC + 100 μg SER	9600 ± 4400*
B	Control with saline + DNP-Ficoll	10,800 ± 5700
	DNP-Ficoll + 100 μg SER	16,400 ± 4100
C	Control with saline + TNP-LPS	18,000 ± 7700
	TNP-LPS + 100 μg SER	18,800 ± 6800

SRBC (in A) or TNP-SRBC (in B and C) was used as target cells to measure the plaque-forming cell response.

* Level of significance at $P<0·05$ by Student's *t*-test.

† PFC, plaque-forming cell.

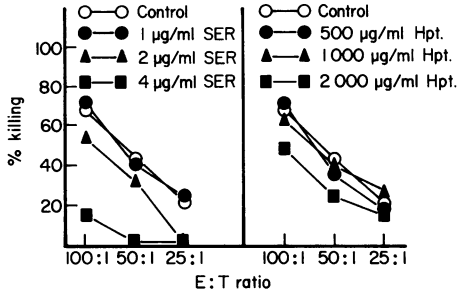

Figure 4. Effect of SER and normal adult haptoglobin on NK cell functions of human PBMC. Purified SER (fraction A) and normal adult haptoglobin (fraction B) were tested on NK cell functions of human PBMC at three different doses as indicated. ^{51}Cr-labelled human myelogenous leukaemic cell line, K562 cell, was used as tumour target cell, and human PBMC served as effector cell. The E:T cell ratios were established at 100:1, 50:1 and 25:1 in 4-hr assay. Same doses of fraction A, B and equivalent volumes of phosphate-buffered saline were added to the tumour target cells in the absence of effector cells. ^{51}Cr released from tumour targets in the absence of effector cells served as spontaneous control counts. Hpt, haptoglobin.

Effect of SER on antibody response to T-dependent and T-independent antigens *in vivo*

Table 1 shows that SER did not significantly inhibit the primary antibody response to T-independent antigens such as DNP-Ficoll or TNP-LPS ($P>0·1$ level) in mice. In contrast to these T-independent antigens, the primary antibody response to T-dependent antigen, sheep red blood cells, was significantly inhibited by SER ($P<0·05$ level). Taken together, the results obtained in Table 1 and Fig. 3 indicate that SER preferentially inhibits the primary antibody response to antigens that are dependent upon T-cell co-operation.

Table 2. Effect of SER and acute-phase proteins on phagocytic index

PBS control		43·8±5·0
C-reactive protein standard serum		56·3±2·5*
Alpha-1-acid glycoprotein		36·8±2·6
Haptoglobin		40·7±4·7
Alpha-1-anti-trypsin		43·3±4·8
IL-1	(20 U/ml)	62·6±5·6*
	(10 U/ml)	64·7±4·4*
	(5 U/ml)	65·0±4·0*
SER	(20 μg/ml)	13·9±8·0†
	(10 μg/ml)	41·1±3·4
	(5 μg/ml)	51·4±3·3

Alpha-1-acid glycoprotein, haptoglobin and alpha-1-anti-trypsin were tested at 20 μg/ml concentrations.

* Significant (at the level of $P<0·05$ by Student's *t*-test) enhancement, compared to control with PBS.

† Significant (at the level of $P<0·05$ by Student's *t*-test) inhibition, compared to PBS control.

Effect of SER on NK cell function

The effects of SER (fraction A) and the normal adult haptoglobin (fraction B) on human NK function were tested at three different concentrations and the NK assay was performed at effector: target cell ratios of 100:1, 50:1 and 25:1. The addition of SER to human tumour target K562 cells alone did not increase the spontaneous release of ^{51}Cr from the labelled target cells, nor did it inhibit the spontaneous proliferation of K562 cells (18-hr incubation, data not shown). Progressive inhibition in human NK function with SER was seen at higher ratios of E:T cells (Fig. 4). Therefore, the inhibitory effect of SER on human NK activity appears to reside on the effector cell level rather than the target tumour cells. Normal adult haptoglobin obtained in fraction B required at least 100 times the concentration of SER before it achieved the same degree of inhibition on NK.

Effect of SER on phagocytic function of polymorphonuclear leucocytes

In the studies performed above we have shown that SER primarily affects immune responses that are dependent upon T-cell function, both in mice and human, and we have already shown that SER interferes with the functions of IL-1 and IL-2 (Oh *et al.*, 1987b). We then examined whether the purified SER could directly interfere with the phagocytic function of normal human granulocytes. As shown in Table 2, the recombinant IL-1 or CRP was able to enhance the phagocytic functions of human granulocytes, while other acute-phase reactants such as alpha-1-acid glycoprotein, haptoglobin or alpha-1-anti-trypsin had no effect. It is interesting that IL-1, even in relatively low doses (5 U/ml), was able to promote significantly the phagocytic functions of PMNs in human peripheral blood. CRP, used as a positive control protein in our study, is known to enhance the phagocytic function of macrophages (Volanakis, 1982). In contrast to CRP and IL-1, SER exhibited dose-dependent inhibition on the phagocytic function of human PMNs. The concentration of SER that achieves a significant degree of inhibition in a phagocytic index is at least 10 times that of the T-dependent mitogenic response. Thus, phagocytosis is a relatively insensitive assay to detect the immunosuppressive property of SER, but this concentration of SER is well within the concentrations that can occur *in vivo* (S. K. Oh, unpublished results). Therefore, it is possible that SER could interfere with the phagocytic function of PMNs *in vivo*, and predispose the patients to microbial infection. The ability of SER to interfere directly with the phagocytic function of PMNs appears to be a unique property of SER, as the other acute-phase reactants were either not inhibitory or enhanced the phagocytic function of PMNs.

In this study, we have shown that affinity-purified SER humoral immunosuppressor primarily interferes with T-cell mediated immunity *in vivo* and *in vitro*. SER immunosuppressor was also found to interfere with the natural immune surveillance mechanisms (NK function) of the host and it may also participate in predisposing the patients to microbial infection. SER that was purified from the body fluids of tumour-bearing patients has been shown to be immunochemically analogous to fetal form of haptoglobin (Oh *et al.*, 1987a). Previous studies from our laboratory have shown that this E-receptor-related immune suppressor, SER, is anti-proliferative to T-dependent mitogenic responses *in vitro* and T-dependent antibody responses *in vivo* in mice (Oh & Moolten, 1981a), and also differentially inhibits the NK cell function of normal lymphocytes (Badger, Oh & Moolten, 1981). However, due to the uncertainty of the exact biochemical identity of the material used in the study, there was a tantalizing question as to whether this multi-faceted inhibitory property of SER could be attributed to other contaminants in the SER preparation used. Therefore, we have utilized an affinity-purified, immunochemically identifiable protein preparation in this study and confirmed our previous findings, indicating that SER does indeed retain down-regulatory function on multiple phases of immune functions both *in vivo* and *in vitro*. SER appears to fit the description of the immune suppressor factor identified earlier in the malignant ascites fluids by Badger *et al.* (1977) and Hess *et al.* (1980). The normal immunosuppressive protein (NIP) described originally by Nelken (1973) also seems to manifest a similar spectrum (Glaser & Nelken, 1972; Nelken *et al.*, 1979; Glaser *et al.*, 1975; Goren & Nelken, 1981) of immunosuppressive properties as our SER. However, it is questionable whether NIP is identical to our SER. First, we have shown previously that the immunosuppressive peptide obtained by acid extraction of malignant ascites is biochemically distinct (Oh & Moolten, 1981b) from the large MW SER immune suppressor. Secondly, their active immunosuppressive peptide of 6000–14,000 MW is retainable on Con A–Sepharose media (Gavison-Goren & Nelken, 1983) while our alpha-1 or alpha-2 subunit of haptoglobin 9000–18,000 MW) does not contain carbohydrate residues (Dobryszycka & Katnik, 1982), thus would not adsorb to any of the lectin affinity media. In fact, our active immunosuppressor moiety, unlike the normal adult haptoglobin (Rademacher & Steele, 1987), does not bind to the Con A affinity media (Oh & Moolten, 1981a).

It is also uncertain whether SER is identical to the immunoregulatory alpha-globulin described by Menzoian *et al.* (1974)

from the Cohn fraction IV of normal human plasma or IRA-like protein identified by Wang *et al.* (1977) from the sera or ascites fluids from cancer patients. The active fraction of IRA protein was believed to reside in the peptide fraction (6000–14,000 MW) (Occhino *et al.*, 1973) and we could not separate an active peptide fragment of a similar MW from SER preparation. Furthermore, if SER is dissociated with 8 M urea or acid, it becomes completely inactive (S. K. Oh, unpublished observation).

Through these studies, we have established clearly the role of SER as a multi-faceted inhibitor of the immune surveillance mechanism of the host and that it is different from NIP or IRA.

It is possible, however, that there is more than one moiety of humoral suppressor with a similar immunosuppressive property. An important point to consider at this juncture is whether any of these immunosuppressor moieties can serve as a useful tool to assess the immunological status of the patients. We have produced a series of monoclonal antibodies to SER as well as to normal adult haptoglobin and have obtained some preliminary results indicating its efficacy in monitoring the immune status of patients who are receiving the autolymphocyte therapy (to be published elsewhere). Therefore, we are now in a position to evaluate the pathophysiological role of SER *in vivo* in human.

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