

Special Article

Human Nonspecific Suppressive Lymphokines

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Since the term "lymphokine" first appeared in print over 20 years ago, a tremendous number of these soluble mediators of the immune system have been described. Within the past few years, many human nonspecific suppressive lymphokines have been identified. This review discusses the historical basis of immunologic suppression and suppressor factors. Later reports describing suppressive human lymphokines are then grouped into four categories: primarily stimulatory lymphokines that also mediate certain suppressive activities, suppressive lymphokines produced during altered states of immunity, suppressive lymphokines produced by exogenously stimulated lymphocytes, and suppressive lymphokines produced by unstimulated lymphocytes. Recent work I have been involved in focusing on the human suppressive lymphokine soluble suppressor factor (SSF) is also discussed.

KEY WORDS: Immunosuppression; lymphokines; suppressive factors; review.

INTRODUCTION

Over the past few years, there has been a tremendous increase in the number of identified human lymphokines. A majority of these lymphokines have been stimulatory or helper factors. However, a large number of suppressive lymphokines have also recently been described. This review examines the historical basis of immunologic suppression and suppressor factors and then discusses a number of different types of suppressive lymphokines. The

review concludes by discussing recent work with the lymphokine soluble suppressor factor (SSF).

HISTORICAL BACKGROUND OF IMMUNOLOGIC SUPPRESSION

The term "lymphokines" was first used by Dumonde *et al.* approximately 20 years ago (1). Lymphokines were defined as "cell-free soluble factors, which are generated during interaction of sensitized lymphocytes with specific antigens." Lymphokines were further differentiated from antibodies as being "expressed without reference to immunological specificity." Dumonde *et al.* demonstrated four functions of lymphocyte-produced soluble factors in a guinea pig model: induction of intradermal inflammation, transformation of lymphocytes, cytopathic action on fibroblast monolayers in culture, and inhibition of migration of peritoneal macrophages.

The cellular basis of suppression of the immune system was also first demonstrated approximately 20 years ago, by Gershon and Kondo (2). In these classic experiments, adult mice were made tolerant to sheep red blood cells (SRBC). Cells from these tolerant mice could be transferred to naive mice (mice not previously exposed to SRBC), and these recipient mice would then also be tolerant to SRBC. This induction of tolerance was shown to be mediated by the transfer of a Lyt 2,3⁺ T-cell subset, later known as T-suppressor cells (T_s). Gershon *et al.* later showed that T_s cells are capable of suppressing immunologic responses by other T cells without the aid of B cells or antibodies (3).

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Merging of the concepts of lymphokines and immunologic suppression was first seen in studies by various investigators including Tada, Benacerraf, and others on antigen-specific suppressive lymphokines. T_s cells were collected from mice that did not respond to or had been made tolerant to a specific antigen, such as keyhole limpet hemocyanin (KLH) or L-glutamine-L-alanine-L-tyrosine (GAT). Cell-free extracts from these T_s cells were prepared by sonication, and these extracts were injected into mice that would normally respond to the specific antigen. These recipient mice then showed decreased immune responses to the specific antigen (4).

These suppressive factors, called T_sF , are thought to be products of T_s -cell subsets stimulated by their specific antigen. All share a number of biochemical properties, including being proteins with molecular weights between 35 and 60 kD, specifically binding the antigen used to stimulate T_s cells to make this factor, and bearing determinants coded by a component of the mouse MHC class II region (H-2) called the I-J region, and acting only in mice strains syngeneic to the strain which produced the factor (4, 5). It has also been reported that the target of T_sF is the $Lyt\ 1,2,3^+ I-J^+$ T-cell subset. T_sF causes the latter cells to become active T_s cells (4-6).

Later research has indicated that there are at least two and possibly three or more levels of antigen-specific T_s cells and T_s -cell factors operating in a complex network of regulation. Initially, it was proposed that antigen stimulated a cyclophosphamide-sensitive class of T_s cells designated T_{s1} (5, 6). These cells produce a factor, T_sF_1 . This factor is a single-chain polypeptide containing an antigen-binding site and MHC-encoded determinants and is not genetically restricted to syngeneic strains. T_sF_1 activates a second class of T-suppressor cells, the cyclophosphamide-resistant T_{s2} cells. T_{s2} cells then produce a second factor, T_sF_2 , which consists of two-polypeptide chains linked by a disulfide bond. One of the chains bears an MHC-encoded determinant; the other appears to contain either an antigen-binding site or an idiotype-specific site. This factor is genetically restricted to function only in syngeneic mice. T_sF_2 may then stimulate one of more other sets of T-suppressor cells (T_{s3}) resulting in suppression of specific effector functions (5, 6). For example, T_sF_2 may stimulate the so-called auxiliary cells which function to suppress contact sensitivity. These cells could then produce

a third suppressor factor (T_sF_3), which would actually mediate immunologic suppression (7).

These various putative T-suppressor factors have yet to be isolated or purified. However, their existence has been called into doubt by difficulties in locating the I-J locus in the murine MHC. Numerous reports have indicated that at least some of these factors interact with antisera to determinants coded for by the K end of the mouse H-2 region (8). These determinants, as discussed above, were designated I-J. Reports map the I-J locus "unambiguously" to the mouse MHC (9). However, molecular mapping of the mouse MHC indicates that the two regions surrounding the I-J region (the I-A and I-E regions) are separated by no more than 3.4 kb and may be separated by less than 1 kb or may even be continuous (10). Thus, it is difficult to envision at least one and possibly two (I-J and I-B) polymorphic regions within such a short span of DNA. If the I-J region does exist, other possible locations include existing outside of the murine I region; having identity with E_{beta} or a fragment of E_{beta} but differing in posttranslational modification and/or conformation; and being produced from differential RNA splicing of a polymorphic RNA encoded by I-A, I-E, or a region between I-A and I-E (10).

SUPPRESSIVE ACTIVITIES OF PREDOMINANTLY STIMULATORY LYMPHOKINES

While a large body of literature exists on antigen-specific suppressive lymphokines in mice, few studies have been performed in humans on this type of factor (11, 12). Most corresponding studies in humans have identified nonspecific suppressive lymphokines. A number of reviews on this topic have been published (13-16). Nonspecific suppressive lymphokines fall into two broad classes: predominantly stimulatory lymphokines which also have suppressive functions and lymphokines which appear to be only suppressive. Interferon (IFN) gamma falls into this first class. IFN-gamma is a product of stimulated T and B cells. In humans, IFN-gamma has been shown to inhibit: lymphocyte proliferation to certain mitogens, antigens, and allogeneic cells (17, 18); certain B-cell functions (19); and the mixed lymphocyte reaction (MLR) (17). Recombinant IFN-gamma has also been reported to induce a brief enhancement of IL-2-stimulated lymphokine-activated killer (LAK) cells after 2 days of *in vitro* incubation followed by a pronounced inhibition of LAK activity after 6 days (20). IFN-

associated suppression may occur with IFN concentrations as low as 10 U/ml (17). Johnson and Blalock (21) have shown that IFN can activate suppressor cells in mice that produce a soluble factor capable of suppressing plaque-forming cell (PFC) responses to SRBC. This suppressor factor may be similar or identical to a well-known suppressor factor, the soluble immune response suppressor (SIRS) (discussed below).

Another predominantly stimulatory factor with suppressive actions is the family of transforming growth factors (TGF). These peptides can induce nonneoplastic cells to express transformed phenotypes. The most thoroughly characterized factor of this family is TGF-beta. TGF-beta is a 25-kD homodimer produced by a number of cell types and capable of suppressing B- and T-cell proliferation and NK-cell cytotoxicity (22). Other suppressive effects of TGF-beta include inhibition of IL-1-induced lymphocyte proliferation. This suppression is not due to a direct IL-1 inhibitor, as discussed below, as the suppression occurs distal to the IL-1 receptor (23).

The mechanism of TGF-beta-mediated suppression is uncertain. TGF-beta does not suppress augmentation of NK cytotoxicity by exogenous IL-2 but does suppress augmentation by exogenous IFN. This suggests that TGF-beta functions by inhibiting enhancement of NK cytotoxicity by endogenous IFN (22). However, TGF-beta can suppress IL-2-driven LAK-cell proliferation and cytotoxicity in a dose-dependent manner at concentrations as low as 5 ng/ml (24). It has also been reported that TGF-beta may exert suppressive activities by down-regulation of lymphocyte cell surface receptors (22).

Other predominantly stimulatory factors that also have limited suppressive abilities include IL-6, which inhibits the growth of certain myeloid leukemic cell lines and breast carcinoma cell lines (25); IL-4, which inhibits IL-2 and lipopolysaccharide (LPS)-mediated stimulation of the production of certain immunoglobulins (26, 27); and connective tissue activating peptide I (CTAP-I), which can indirectly mediate immunosuppression by stimulating production of prostaglandin E₂ (28).

SUPPRESSIVE LYMPHOKINES PRODUCED IN STATES OF ALTERED IMMUNITY

A number of lymphokines have been described as being produced primarily or solely in disease states

or other states characterized by altered immune responses. Linker-Israeli and Casteel (29) have described factors produced by unstimulated peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). These factors, termed SF PBM, suppressed production of IL-2 by PHA-stimulated PBMC from healthy subjects or SLE patients and IL-2 production by Jurkat cells, a constitutive IL-2-producing cell line. Both monocytes and CD8⁺ lymphocytes were necessary to produce this suppressive activity. SF PBM did not inhibit proliferation of IL-2-dependent or -independent cell lines and had no IFN activity. These factors are sensitive to acid (pH 2), 56°C, and trypsin. Two molecular weight ranges of SF PBM activity were detected by gel filtration, <150 kD (fraction II) and 40 to 60 kD (fraction IV). Fraction II mediated suppression in a manner similar to unseparated SF PBM, while fraction IV was active only when monocytes were present. This suggests that fraction IV requires monocyte processing for activity such as the suppressive lymphokine SIRS, discussed below; however, 2-mercaptoethanol, an inhibitor of SIRS activity, had no effect on either fraction.

Pregnancy, while clearly not a disease state, is characterized by alterations in immunity. Nagaoki *et al.* (30) have shown that pokeweed mitogen (PWM)-stimulated cord blood T cells produce a dialyzable factor capable of suppressing the generation of plasma cells by PWM stimulated adult peripheral blood lymphocytes (PBL). Cord non-T cells, adult T cells, or adult non-T cells were incapable of producing this suppressive factor. Development of suppressive activity took at least 12 hr following PWM stimulation and was no longer produced after 48 hr poststimulation. This factor, named cord T cell-derived suppressive factor (CTSF), is stable at 56°C for 30 min but is inactivated at by treatment at 80°C for 10 min (31). Production of CTSF activity was abrogated by irradiating cord T cells with 1000 rads (30).

Williams and Korsmeyer (32) have studied suppression mediated by supernates from Con A-activated lymphocytes in two other immunologically altered states. Supernates from a subset of healthy individuals were capable of suppressing the MLR. A greater proportion of supernates from cord blood samples or from subjects with common variable immunodeficiency showed suppression of the MLR. This suppressive activity was stable at 56°C but inactivated at 70°C. Suppressive activity was

disrupted by treatment at pH 2.0 or with chymotrypsin but was resistant to neuraminidase, RNase, or DNase. This activity may be identical to other suppressive factors discussed herein, but definitive purification and characterization have not been reported.

Several investigators have examined suppressive lymphokines produced in human immunodeficiency virus (HIV)-infected individuals. Laurence *et al.* (33) have shown that supernates from cultured PBMC of patients with the acquired immune deficiency syndrome (AIDS) or its prodrome were capable of suppressing spontaneous and PWM-stimulated differentiation of B cells as well as antigen-mediated T-cell transformation, while no such suppressive activity was found in comparable supernates of healthy controls. These supernates did not affect T cell-independent immune responses. Suppressing activity was produced by the interaction of T cells with adherent cells. T cells from AIDS patients, but not from healthy controls, in collaboration with adherent cells from healthy control individuals were also capable of producing this activity. This activity was reversed by 2-mercaptoethanol, suggesting that the activity may be similar to the soluble immune response suppressor (SIRS), discussed below.

Henderson *et al.* (34) have examined a suppressive factor(s) produced by healthy individuals in response to ultraviolet-irradiated HIV (uv-HIV). Cultures of healthy PBMC exposed to uv-HIV in the presence of PHA produce supernates capable of suppressing PHA-induced proliferation in other PBMC cultures. Supernates from PBMC cultures either untreated or treated with only PHA did not mediate this suppression. Suppression was not due to the presence of the uv-irradiated HIV or residual HIV proteins. This suppressive activity is sensitive to heat (1 hr at 56°C) and trypsin. It was suggested that this immunosuppression may be due to killing of CD4⁺ cells.

SUPPRESSIVE LYMPHOKINES PRODUCED BY EXOGENOUSLY-STIMULATED LYMPHOCYTES

A number of factors have been described that are produced by concanavalin A (Con A)- or pokeweed mitogen (PWM)-activated lymphocytes or lymphocyte subsets. One of the first sets of Con A-activated factors to be characterized is the family of migration inhibitory factors: fibroblast migration inhibiting factor (FIF), leukocyte migration inhibiting

factor (LIF), and macrophage migration inhibiting factor (MIF). These factors are all similar in function, inhibiting migration of their target cells specifically without inhibiting other functions of these cells.

MIF has both stimulator and inhibitor activities, as MIF-mediated inhibition of monocyte/macrophage migration mediated by MIF is also correlated with cutaneous delayed-type hypersensitivity (DTH). MIF is actually a group of peptides, ranging in molecular weight from 23 to 65 kD and in *pI* from 2.4 to 5.6. These differences are not due to alterations in glycosylation, as tunicamycin does not convert one species into another (35). Characteristics of MIF include stability at 56°C; inhibition by L-fucose, L-rhamnose, and other 5-methylpentoses; and binding to a cell surface glycolipid receptor containing both fucose and sialic acid (35, 36). MIF can apparently be produced by either T or B cells alone, without the influence of the other subset of lymphocytes (37). Abnormalities in the production of MIF may play a role in disease symptomatology, as lymphocytes from subjects with DiGeorge's syndrome do not produce MIF (38). This also suggests that at least small numbers of T cells must be present with B cells for MIF production.

LIF has an approximately molecular weight of 69 kD and is produced by T and B cells following antigen or mitogen stimulation. LIF, similar to MIF, has both suppressive and stimulatory activities, as LIF-mediated lymphocyte migration inhibition occurs in concert with cellular hypersensitivity (37). LIF activity is inhibited by *N*-acetyl-D-glucosamine (39). LIF appears to function as both an esterase and a protease as demonstrated by its susceptibility to phenylmethylsulfonyl fluoride (PMSF), an irreversible enzyme inhibitor (40). Treatment of PMN with LIF elevates intracellular cGMP levels (41), while the production of LIF is inhibited by agents which increase intracellular cAMP (42).

FIF is an approximately 34-kD molecular weight factor that, as the name suggests, specifically inhibits the migration of fibroblasts. FIF activity is disrupted by incubation at 80°C, and treatment with trypsin, chymotrypsin, and neuraminidase, and is blocked by L-xylose, *N*-acetyl-D-glucosamine, D-mannose, L-rhamnose, and alpha-methyl-D-mannoside but not by L-fucose (39). A T-cell migration inhibitory factor (TIF) has also been described (43). This factor is produced by cultures of PHA-treated T cells and inhibits migration of human T

lymphocytes. TIF activity has a molecular weight of 1 to 10 kD, an isoelectric point of 3.1, and can be separated from MIF by polyacrylamide gel electrophoresis. TIF activity is resistant to trypsin, chymotrypsin, and neuraminidase but, like LIF, is inhibited by PMSF.

Soluble immune suppressor supernates (SISS) consists of two factors also produced by Con A-stimulated lymphocytes: SISS-T and SISS-B (36). SISS-T inhibits mitogen- and antigen-stimulated T-cell proliferation. Characteristics of SISS-T include a molecular weight of 30 to 45 kD; partial inactivation at 56°C, complete inactivation at 80°C; being unaffected by exposure to acid or base; partial inhibition by *N*-acetyl-D-glucosamine (NAG); production by lymphocytes resistant to 2000 R but not to 6000 R; and binding to the same cell surface receptors as the lectin wheat germ agglutinin (WGA) and *Agaricus bisporus* lectin (36). The latter finding is especially interesting, as WGA has been shown to bind to IL-2 receptors and interfere with maintaining high-affinity IL-2 binding sites on activated T cells, resulting in decreased peripheral blood mononuclear-cell (PBMC) proliferation (44).

SISS-B has been clearly shown to differ from SISS-T. Functionally, SISS-B inhibits *in vitro* immunoglobulin (Ig) production by activated B cells. SISS-B has an apparent molecular weight of 60 to 80 kD and is stable at pH 2.5 but is inactivated at 56°C. The activity of SISS-B is inhibited by L-rhamnose but not affected by NAG. SISS-B is produced by highly purified T cells (45). CTC-SISS-B, a suppressive factor produced by an IL-2-dependent suppressor T-cell line, appears to be similar or identical to SISS-B (46). CTC-SISS-B has been shown to suppress Ig production by directly interacting with activated B-cells, rather than via an indirect interaction with T cells or monocytes.

Another suppressor factor produced by Con A-activated lymphocytes is the soluble immune suppressor factor (SISF) (47). SISF also binds to cells at the same surface receptor(s) recognized by WGA. A recent report states that patients with a variety of cancers show increased numbers of peripheral blood lymphocyte (PBL) expressing surface receptors for WGA. These unique lymphocytes decrease in number in patients who undergo curative resection of tumors or who respond to immunotherapy but not in patients who receive noncurative resection or who do not respond to immunotherapy, suggesting that SISF may play a

role in the immunosuppression evidenced in many cancers (47). As SISF has not been well characterized, it may be identical to other lymphokines such as SISS-T, which also binds to the cell surface WGA receptor.

Inhibitor of DNA synthesis (IDS) is another inhibitory lymphokine produced by Con A-stimulated lymphocytes. As the name suggests, IDS inhibits DNA synthesis by human peripheral blood lymphocytes. IDS suppresses lymphocyte proliferation in response to antigens and lectins (48). Human IDS has a *pI* of 3.4 and a molecular weight of 20 kD; however, IDS is usually found in an 80-kD tetrameric form. IDS' activity is inhibited by trypsin but is resistant to heat, RNase, and DNase. Periodate treatment also inhibits IDS-mediated suppression, suggesting that IDS is a glycoprotein and the carbohydrate moiety is necessary for suppressive functions (49). The mechanism of IDS-mediated suppression appears to be activation of adenylate cyclase activity in target cells (50). Suppression mediated by IDS is specific for DNA synthesis. IDS inhibits DNA synthesis in PHA-stimulated PBMC but has negligible effects on protein and RNA synthesis in these cells, including production of IL-2, IL-2 receptor, and transferrin receptor. This further suggests that the mechanism of action of IDS is IL-2 independent (48).

Pancre *et al.* (51, 52) have described a platelet activity-suppressive lymphokine (PASL) produced by Con A or antigenic stimulation of normal human CD8⁺ lymphocytes. PASL is capable of suppressing IgE-dependent platelet cytotoxicity against *Schistosoma mansoni* larvae and production of oxygen metabolites by IgE-coated platelets. PASL is also produced by T lymphocytes from *S. mansoni*-infected humans when stimulated by *S. mansoni* antigens. This factor has a molecular weight of 15 to 20 kD and a *pI* of 4.6. PASL is heat and acid stable and sensitive to trypsin and proteinase K but is not affected by neuraminidase treatment. Indomethacin does not affect PASL production. PASL appears to act directly on platelets, suggesting that it interacts with a platelet membrane surface receptor. PASL produced by humans is capable of inhibiting rat platelet cytotoxicity and rat PASL can suppress corresponding functions of humans.

PWM-stimulated CD4⁺ cells have been reported to produce T-suppressor growth factor (T_sGF) (53). This approximately 8-kD factor is necessary for the proliferation and differentiation of CD8⁺ lymphocytes and functions by inducing IL-2 receptor

expression on CD8⁺ cells. CD8⁺ cells stimulated by IL-2 plus T_sGF demonstrate either the CD11⁺ (suppressor effector) or the CD45R⁺ (suppressor inducer) phenotypes and are CD28⁻ (cytotoxic T cell, T_c, phenotype). Prostaglandin E₂ (PGE₂) and IFN-gamma may also be necessary for differentiation of certain CD8⁺ subsets.

Another suppressive factor produced by stimulated human lymphocytes is T cell-specific suppressive activity (TSRA), a factor produced by T lymphocytes stimulated by a soluble suppressor activating factor (SAF) (54). SAF is produced by a 6-thioguanine-resistant mutant of the human T-cell line, CEM. TSRA is capable of suppressing mitogen-activated T-cell proliferation, T_c generation, and PWM-stimulated PFC responses. SAF shows similar functional activities to TRSA but TRSA is 50- to 500-fold more potent. Some evidence exists that TSRA is an activated form of SAF, paralleling the SIRS system (discussed below). Release of TSRA by SAF-stimulated lymphocytes appears to be partially inhibited in rheumatoid arthritis patients (54).

Soluble inhibitory factor (SIF) is a unique factor produced by T cells stimulated by tetanus toxoid or by MLR with cells from unrelated donors (55, 56). SIF appears to be produced by dividing T cells in proportion to their rate of division. SIF mediates inhibition of DNA synthesis and proliferation of PHA-stimulated PBL as measured by decreased ³H-thymidine uptake, as well as inhibition of Ab production and PFC responses (55, 56) and inhibition of ³H-thymidine uptake in the MLR (57). Characterization of SIF has revealed that this factor has two components, a carrier protein and a polar lipid. Immunosuppression is mediated by the lipid moiety, which is termed lipid suppressor substance (LSS). LSS and the protein are apparently bound by hydrophobic interactions, and are separated by dialysis against hypotonic solutions. LSS activity is stable at 56°C for 30 min but is destroyed by 70°C for 15 min. LSS is a polar lipid, possibly a glycolipid containing an amine group. However, LSS does not appear to be identical to common gangliosides (56). The protein moiety, which does not mediate immunosuppression, has a molecular weight between 100 and 150 kD (57).

Intact SIF activates a suppressor-cell population capable of inhibiting antigen- and mitogen-induced thymidine incorporation. These suppressor cells are not affected by the DNA synthesis inhibitor, mitomycin C, indicating that their suppressor function

does not require DNA synthesis. LSS by itself, however, does not appear to be capable of suppressor-cell induction. LSS may function by altering lymphocyte membrane function or fluidity (57).

SIF has also been identified in normal human sera and found to be present at very high concentrations in serum from a patient with an unusual cutaneous lymphoma (57). SIF is also produced by cultures of multinucleated giant cells obtained from the villous surfaces of normal human placentae (58).

One of the most extensively described suppressor factors produced by stimulated lymphocytes is the soluble immune response suppressor (SIRS). Information on SIRS, both published and unpublished, has recently been reviewed by Aune (59). Much of the work on SIRS has been performed in the murine system, although recent work has extended some of these observations to human immunology. SIRS is produced in a precursor form by Con A-stimulated murine Lyt 1⁻2⁺ T cells. This precursor is oxidized to the active (or more active) state by macrophages. To mediate suppression, SIRS-containing supernates must be added at the initiation of cell culture; however, SIRS-containing supernates cocultured with macrophages (to produce the oxidized form of SIRS) are capable of suppressing lymphoid cells when added several days after the start of culture (60).

SIRS is capable of suppressing primary and secondary IgM and IgG responses *in vitro* to T cell-dependent and -independent antigens, DNA synthesis responses of lymphocytes to mitogens and alloantigens, and DNA synthesis and cell division by certain tumor-cell lines (60). SIRS-mediated suppression is inhibited by various compounds including catalase, 2-mercaptoethanol (2-ME), and peroxidase substrates. It is thought that these compounds inhibit the oxidation of the SIRS precursor by macrophages (60).

SIRS has undergone partial biochemical characterization. The molecular weight of murine SIRS was initially determined to be 45 to 55 kD (60). Later work with a murine SIRS-producing hybridoma yielded a factor with a molecular weight of 21.5 kDa that decreased to a molecular weight of 10 to 12 kD in high-ionic strength buffers (61). Three isoforms of murine SIRS have been described, with *pI* values of 5.0, 6.0, and 7.0 (62).

Recently, a murine SIRS has been partially sequenced. Antisera produced against a peptide consisting of the initial 21 N-terminal amino acids of SIRS can block suppressive activity mediated by

intact SIRS (63). Two mRNAs have been isolated which produce SIRS activity in a cell-free translation system. By velocity sedimentation, one mRNA is 21 to 22 S, while the other is 25 S. The former produces a product of molecular weight 7 to 8 kD, while the latter produced two products: one of 13 to 14 kD and one of 7 to 8 kD. Both products had SIRS-like activity, and this activity was blocked by inhibitors of SIRS (64). The basis for the different apparent molecular weights of SIRS is unknown.

Human SIRS had been previously described to have a molecular weight of approximately 130 kD (65); however, a later report indicated that the molecular weight of human SIRS in high-ionic strength and low-pH buffers was 15 kD (66). Human SIRS has a *pI* of 7.0 (59) and is inactivated at pH 2 but not pH 4 (65).

The mechanism of action of SIRS is under investigation. In PFC assays, SIRS does not appear to inhibit Ig synthesis but, rather, interferes with Ig processing and/or secretion (66). It has been suggested that SIRS mediates this immunosuppression by causing oxidation of intracellular low molecular weight thiols and protein sulfhydryls (59). One target for this oxidation is tubulin; SIRS is capable of inhibiting assembly of microtubule protein in a log concentration-dependent manner (67).

IFN-associated immunosuppression may actually be mediated by SIRS. Murine IFN-beta activates a subset of suppressor lymphocytes (Lyt 1⁻2⁺) that suppress plaque-forming cell (PFC) responses to sheep red blood cells (SRBC). This lymphocyte subset, when activated by IFN-beta or Con A, produces supernates which show many properties similar to SIRS (60).

A similar system has been described in humans for the PFC response mediated by PWM-stimulated PBMC. This response can be suppressed by CD8⁺ lymphocytes activated by Con A, IFN-alpha A, or IFN-gamma. Suppression mediated by these cells or by their supernates can be blocked by inhibitors of SIRS activity, including levamisole, catalase, 2-ME, and ascorbic acid. This suppressive activity showed kinetics similar to those described for murine SIRS (65). Kaufman *et al.* (68) also reported that supernates from Con A-treated human lymphocytes are capable of suppressing allogeneic and syngeneic MLR without causing cytotoxicity. This suppression was described as being "SIRS-like," although the suppressive activity was not further characterized.

SIRS may also play a role in disease states. It has been reported that SIRS levels are elevated in schistosomiasis and in the nephrotic syndrome (59). Whether this elevation is a cause or a symptom of these states is unknown.

Warrington (69) described a factor similar to SIRS produced by con A-activated human T cells, called T cell-suppressor factor (T-SF). T-SF induces macrophages to produce macrophage-derived suppressor factor (macrophage-SF) in the absence of any *de novo* protein synthesis. Both of these factors can suppress polyclonal B cell-activated Ig production without affecting B- or T-cell proliferation and can mediate this suppression in the absence of T-cells. T-SF activity is inhibited by 2-ME and L-rhamnose. While the activity of macrophage-SF is inhibited by L-rhamnose, only its production appears to be inhibited by 2-ME. T-SF must be added at the start of culture to mediate suppression, while macrophage-SF can be added later. T-SF has a molecular weight of approximately 20 kD, while macrophage-SF appears to be a complex with molecular weight >110 kD that dissociates into a 20-kD form in high-ionic strength buffers (69).

Many properties of T-SF and macrophage-SF suggest that these molecules are identical to SIRS. However, there are some major differences in their actions. SIRS suppresses cell replication, while T-SF and macrophage-SF do not. T-SF cannot be activated to form macrophage-SF by peroxide, while SIRS can be so activated (69). Whether these two factors are identical to SIRS has yet to be determined.

Cytokine synthesis inhibitory factor (CSIF), a recently reported lymphokine now designated as IL-10, is also produced by Con A-stimulated T cells (70). Murine IL-10 is produced by one class of long-term mouse T-cell clones, T_H2 clones, and inhibits many of the functions of another class of clones, T_H1. This includes inhibition of the production of IFN-gamma and partial inhibition of the production of other cytokines (70). IL-10 has no suppressive effects on cytokine production by T_H2 clones and does not affect viability or proliferation of T_H1 clones (71). Inhibition of IFN-gamma production is due to inhibition of mRNA synthesis and is not due to alterations in IFN-gamma secretion (71). Inhibition of other cytokines produced by T_H1 clones, including IL-2, lymphotoxin/tumor necrosis factor (LT/TNF), IL-3, and granulocyte/macrophage colony-stimulating factor (GM-CSF), is spo-

radic; their synthesis is inhibited only in some instances (71).

IL-10 is not capable of suppressing early cytokine synthesis. Thus it is not effective during the first 8.5 hr following stimulation of clones of T_H1 cells; only synthesis after this period is suppressed. Further, IL-10-mediated suppression is seen only in the presence of antigen-presenting cells (APC). One hypothesis is that IL-10 is modified in order to mediate suppression or IL-10 induces the synthesis of a second lymphokine which mediates suppression (71). This could be similar to the mechanism of action of SIRS, described above, where the initial "prolymphokine" is modified by macrophages to form a more active moiety.

IL-10 may also be produced in smaller amounts by unstimulated T_H2 clones, but reports discussing this are contradictory (70). DNA coding IL-10 has recently been sequenced and shown to have homology with the Epstein-Barr virus gene BCRF1 (70).

Other suppressive activities yet to be fully characterized, but which may be similar or identical to factors described above, include a pair of suppressor factors, SFR and SFNR, produced by allosensitized cells (72); a factor produced by PWM-stimulated suppressor T cells capable of suppressing autologous and allogeneic IgG, IgA, and IgM synthesis (73); and a factor produced by PWM and IgG immune complex stimulated T lymphocytes bearing receptors for IgG (T_G cells) that suppresses PWM-induced B-cell differentiation (74).

SUPPRESSIVE LYMPHOKINES PRODUCED BY UNSTIMULATED LYMPHOCYTES

A small number of factors have been described that are produced by unstimulated lymphocytes. The term "unstimulated" is somewhat of a misnomer, as even lymphocytes in isolation are constantly being exposed to autologous HLA antigens, locally released mediators, and other endogenous molecules. Hence, unstimulated refers here to the lack of addition of any exogenous stimulatory molecules. As noted above, IL-10 may be produced in the absence of any stimulation (70). Small amounts of SIF may also be produced by unstimulated T cells (65). Mortari and Singhal (75) have described a factor produced constitutively by a suppressor-enriched population of normal human bone marrow cells called bone marrow-derived suppressor factor (BDSF). BDSF suppresses *in vitro* primary anti-

body responses and lectin-induced lymphocyte proliferation. BDSF has a molecular weight less than 1.5 kD and contains a lipid moiety, but its production is indomethacin resistant. The cell type responsible for the production of BDSF is HNK-1⁺, but it does not bear surface markers of T or B cells or macrophages.

Lederman *et al.* (76) have also described a factor produced by unstimulated lymphocytes called immune response suppressor factor (IRSF). This factor is produced by a nonadherent cell population after 4 hr of culture *in vitro*. IRSF does not affect cell viability, and its release is not inhibited by indomethacin. IRSF is heat stable and is partially dialyzable through 12-kD exclusion size membrane. No further characterization of this factor has been performed, and its relation to other factors, including SSF (discussed below) is unknown.

Soluble receptors for Fc portions of IgG (FcR) have been reported as being released from unstimulated human PBL. Although not commonly thought of as lymphokines, FcR fulfill the definition by being non-Ab, immunoregulatory molecules produced by lymphocytes. FcR are capable of inhibiting the differentiation of PWM- or *Nocardia opaca*-stimulated peripheral B cells into Ig-secreting plasma cells without affecting cell viability or ³H-thymidine incorporation (77). Inhibition is dose dependent. Human FcR are thought to be analogues of murine immunoglobulin binding factors (IBF), glycoproteins which bind Fc portions of IgG and suppress the PFC response to SRBC (78) (79).

Work in our laboratory has focused on the suppressive lymphokine soluble suppressor factor (SSF). SSF is lymphokine produced by unstimulated PBL in serum-free medium (80). SSF induction is apparently a consequence of the autologous mixed lymphocyte reaction, as it is produced by neither T cells nor B cells alone. However, adherent cells are not required for its production. SSF is generated only in serum-free medium, as the presence of serum inhibits its production. Biological actions of SSF include suppression of polyclonal B-cell activation, proliferative responses of normal PBL in responses to mitogen or alloantigens, and natural killer (NK) and antibody-dependent cellular cytotoxicity (ADCC) (81, 82).

The mechanism of SSF-mediated suppression is uncertain but is not due to a cytotoxic effect (81). Binding of SSF to cell surfaces appears to involve a carbohydrate moiety, as SSF-mediated suppression of NK cytotoxicity can be inhibited in a dose-

dependent manner by L-fucose (Fuc) or D-galactose (Gal) (83). This inhibition appears to be due to binding of SSF to the monosaccharide, rather than the monosaccharide blocking a site on the cell surface. For the inhibition of SSF-mediated suppression, monosaccharides must be present within 24 hr following the addition of SSF to effector-cell cultures; after 24 hr, the addition of monosaccharides does not affect suppression.

Suppression mediated by SSF may be limited to preventing stimulation of lymphocytes rather than suppressing lymphocytes already stimulated. In the one-way MLR, SSF was capable of mediating suppression when added during the first 4 days of culture but not when added on day 5 or 6 (81). This suggests that only early events in the MLR can be suppressed by SSF.

Investigations into the interaction of SSF with other lymphokines has begun. The immune modulator IFN-alpha is capable of partially reversing SSF-mediated suppression of NK cytotoxicity (82). However, recent experiments indicate that IFN can also stimulate production of SSF activity (84). This paradoxical activity of IFN may serve as a feedback inhibition mechanism within the AMLR, preventing overstimulation and possible subsequent autoimmune reactions. IL-2 is also capable of reversing SSF-mediated suppression. In contrast to the actions of IFN, however, IL-2 appears to be able to suppress production of SSF (80).

The biochemical characterization of SSF has been partially completed. SSF activity appears in the fraction <20 kD when separated by membrane ultrafiltration (82). On gel filtration, activity appears in two peaks: one between the 25-kD and the 13.7-kD markers and the second below the 13.7-kD marker. The higher molecular weight peak showed a majority of the biological activity (82). SSF is heat stable at 56°C for 30 min, but loss of suppressor activity occurs after incubation at 70°C for 30 min (81). SSF is sensitive to the proteolytic enzymes trypsin and chymotrypsin, suggesting that it is, at least in part, a polypeptide (80). Acidic conditions slightly decrease SSF's activity, while its activity is totally abrogated following treatment at basic pH (80). SSF also appears to be glycosylated, as SSF activity is specifically removed from SSF-containing supernates by lentil lectin (from *Lens culinaris*). SSF activity can be recovered following elution of lectin-bound material by 0.1 M mannose (unpublished observation).

SSF has recently been isolated in our laboratory (85). Following controlled-pore glass (CPG) chromatography, SSF activity is found in a high-pH (0.1 M Tris, pH 8.5) elution. The suppressive activity in this elution has a molecular weight less than 30 kD, is sensitive to proteases, and is inhibited by anti-SSF monoclonal antibody (mAb), all identical to SSF activity in crude supernates.

On polyacrylamide gel electrophoresis, this CPG elution reveals one major band with a molecular weight of approximately 20 kD. Electroelution of this band produces material with SSF activity. This activity is also inhibited by anti-SSF mAb (85). Isoelectric focusing (IEF) gel electrophoresis of the polyacrylamide gel eluate reveals two bands, at *pI* values of 8.2 and 8.4. Upon electroeluting, the eluates from either band are capable of mediating SSF-like suppression, and this suppression is inhibited by anti-SSF mAb. We therefore conclude that this purified material is SSF. The two *pI* values may be due to alteration caused during the harsh purification process or to native SSF having two distinct isoforms.

SSF does not appear to be identical to other previously isolated soluble mediators. Antibodies to IFN-gamma, IL-1, IL-2, TGF-beta, platelet factor 4 (PF4), and connective tissue activating peptide III (CTAP-III) do not affect SSF-mediated suppression of NK cytotoxicity (85). Antibody to tumor necrosis factor (TNF) does partially inhibit SSF-mediated suppression; however, even at a very high concentration (100 U/ml), TNF is incapable of mediating suppression of NK cytotoxicity (85). We therefore conclude that SSF and TNF are distinct moieties but may well share sequence homology to some degree.

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 (unpublished observation). Production of SSF is unaffected by preirradiation of lymphocytes with up to 2000 rads (80), suggesting that SSF may be produced by a unique radioresistant subset of lymphocytes of the suppressor phenotype.

Nair *et al.* (86) have described a suppressor factor produced by histamine-stimulated PBL (histamine-induced soluble suppressor factor, HISSF). Like SSF, this HISSF is capable of suppressing NK cytotoxicity, and this suppression can be partially reversed by IFN-alpha or IL-2. This factor shows at

least partial sequence homology to SSF, as a mAb generated against HISSF is capable of neutralizing the activity of SSF (unpublished observation). Thus it remains to be determined if histamine can up-regulate SSF production/activity or whether SSF and HISSF are two distinct moieties.

CONCLUSION

Our understanding of the role of lymphokines in immunosuppression has greatly increased over the past few years. It is now evident that suppressive lymphokines, or lymphokines with certain suppressive functions, are vital for the homeostasis of the immune system and are probably involved in the etiology and pathogenesis of a number of disease states. Unfortunately, there has been little characterization of most of these lymphokines beyond initial descriptions. To further our understanding of the complex interactions within immune responses, in both in normal states and diseased or altered states, a more rigorous examination of various suppressive activities is necessary. Similarities or identities between different mediators can then be determined, and a more integrated picture of immune regulation may be revealed.

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