

Okadaic Acid-Like Toxin in Systemic Lupus Erythematosus Patients: Hypothesis for Toxin-Induced Pathology, Immune Dysregulation, and Transactivation of Herpesviruses

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Abstract — Preliminary evidence suggests there is a toxin in the sera of systemic lupus erythematosus patients which reacts with a commercial enzyme-linked immunosorbent assay kit for the detection of the marine toxin, okadaic acid. Data is presented which supports the hypothesis that an okadaic acid-like toxin may be the principle agent of lymphocyte dysregulation in systemic lupus erythematosus and other immune-dysregulated states.

The okadaic acid-like toxin can produce the specific abnormalities in T-lymphocyte phenotype and function typical of systemic lupus erythematosus, principally through its ability to inhibit serine/threonine phosphatases necessary for secondary signalling processes, and through its ability to inhibit calcium which is crucial to protein kinase C-mediated signalling of T-lymphocytes. The disruption probably occurs through the protein tyrosine kinase p56^{lck} pathway crucial for IL-2. Additionally, the toxin's ability to disrupt voltage-sensitive ion channels in cell membranes may be responsible for the multi-organ pathology observed in systemic lupus erythematosus patients, particularly neurological, cardiac and nephritic.

Data from a different study conducted by the author suggests that latent and persistent viruses are reactivated in active lupus. This activation could be the result of the toxin's ability to act as an immune modulator, or its ability to act as a transactivating factor.

Introduction

Systemic lupus erythematosus (SLE) is a disease of immune dysregulation involving lymphocytes. In brief, the immune profile is activated B cells (for review see 1); decreased CD4 naive suppressor-inducer T cells and CD8 cytotoxic/suppressor T cells (2–4);

impaired natural killer (NK) cell function (for review see 5); defective CD2 T cell activation (6); decreased expression of CD3 (part of T cell receptor complex) on T cells (3,7); defective CD3 signalling (8); and altered CD4:CD8 ratio (3,9,10). In addition, SLE is characterized by defective cytokine production and utilization (11–14).

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Although the etiological agent of SLE has not been isolated, the application of recent advances in virology and immunology from acquired immune deficiency syndrome (AIDS) research may lead to the discovery of a putative organism capable of SLE immune dysregulation.

Although inherited factors have overshadowed the search for an exogenous agent as the cause of SLE, a likely candidate is an organism which produces a factor capable of altering immunity.

It is known that bacterial toxins suppress or enhance T-cell phenotypes and antibody formation (15-17). Also, endotoxin was long ago postulated as a B-cell-activating substance which might induce autoimmunity (18).

By applying these concepts to the study of SLE, an hypothesis can be constructed around the concept that the factor which alters immunity in SLE patients is a toxin. Preliminary data support this conclusion. It is also suggested that, in addition to disrupting immunity, the toxin reactivates herpesviruses in SLE patients. Reactivated viruses, principally herpes, in active SLE patients have been confirmed in a separate manuscript by the author (submitted), and a possible relationship to the toxin is presented.

Hypothesis

Evidence for a toxin in systemic lupus erythematosus patients which cross-reacts with okadaic acid in enzyme-linked immunosorbent assay

Okadaic acid (OA) is a toxic polyether which has been derived from the marine dinoflagellate *Prorocentrum* (19). It is among a group of marine toxins known to cause fish and shellfish poisoning in mammals (20). The marine toxins cause symptoms ranging from diarrhea to death.

In a preliminary experiment utilizing a commercial competition enzyme-linked immunosorbent assay (ELISA) (Rougier Bio-Tech), it was determined that the sera of SLE patients contains a substance which reacts with OA anti-idiotypic antibody (21). The SLE sera utilized in the experiment was from both active and inactive SLE patients.

Mechanism of toxin-induced multi-organ pathology

Marine toxins exert their toxic effect through disrupting voltage-sensitive ion channels in cell membranes. Ciguatoxins and brevetoxins bind neuronal sodium channels (22). OA lowers Ca^{2+} influx, and suppresses release of Ca^{2+} from intracellular Ca^{2+} stores (23). Novelli et al reported that domoic acid (which causes headache, confusion and memory loss)

may disrupt Mg^{2+} channels in neuron cultures, leading to the potentiation of excitotoxic amino acids (24). Molgo et al have reported that ciguatoxin disrupts acetylcholine release through Na (25). Betz and Henkel have demonstrated that OA can reduce acetylcholine response in frog nerve (26). Maitotoxin can regulate Ca channels to allow influx (27), leading to inhibition of parathyroid hormone in bovine cells (28), and the influx causes depletion of adenosine triphosphate (ATP) leading to myocardial cell death in rat cardiomyocytes (29). In one study, quinidine (Na channel blocker) and verapamil (Ca channel blocker) were able to inhibit palytoxin-induced catecholamine secretion caused by its ability to cause Na and Ca influx into bovine adrenal cells (30).

It was recently reported that a researcher working in proximity to tanks of dinoflagellates (which can produce marine toxins) suffered extreme neurological impairment, 'wildly erratic' heartbeat, blood pressure, and other symptoms (31). Low levels of toxins could result in the less dramatic, yet similar, symptoms in SLE patients (for review see 32,33).

Clearly, toxin ionophores have the capacity to alter critical cellular processes. A toxin in SLE patients could contribute to multi-organ involvement, particularly in relation to ion-channel-dependent kidney, heart, lung and brain function. It is interesting that quinine (derivatives of which are used in the treatment of SLE) is a voltage-dependent K channel blocker which inhibits Ca-induced K loss (34). While quinine is not known to affect Ca channels, it is Ca-dependent. In an experiment with frog lens, it reversed hydrogen peroxide-induced decreased membrane resistance caused by suspected increased Ca influx (35). It has been suggested that hydroxychloroquine's efficacy in the treatment of SLE may be due to its ability to alter antigen processing (36). Another mechanism of action may be its ability to alter Ca ion exchange. Some toxins disrupt ion exchange by binding membrane channels (37,38); others create pores in membranes (39,40). If cellular membranes in SLE patients were bound by a toxin, autoantibodies would be expected to form. Autoantibodies to voltage-gated Ca channels have been reported in SLE patients (40).

Involvement of an okadaic acid-like toxin in disrupting immunity

Okadaic acid directly affects the production of cytokines consistent with aberrant patterns detected in systemic lupus erythematosus patients

Interleukin 1. In a study of active SLE patients, Linker-Israeli et al found that, although elevated

levels of interleukin 1 (IL-1) messenger ribonucleic acid (mRNA) were detected in peripheral blood mononuclear cells (PBMCs), there was no indication that IL-1 was present (13).

This appears consistent with the differential effect of OA on the production of IL-1 demonstrated by Hokama et al (41). OA in concentrations of 0.01–5.0 μ M added to PBMCs stimulated the production of IL-1. However, concentrations of 1.0 μ M or greater significantly inhibited IL-1. It is suggested that the phenomenon observed in the Linker-Israeli study is a reflection of an inhibitory effect of an OA-like toxin on IL-1.

Interleukin 6. SLE patients exhibit elevated levels of interleukin 6 (IL-6) (14). This is related to polyclonal B-cell activation and anti-deoxyribonucleic acid (DNA) autoantibodies (43). OA induces IL-6 (44).

Tumor necrosis factor. SLE patients exhibit significantly elevated soluble tumor necrosis factor receptors (sTNFRs) (13). These soluble receptors are reported to be more strongly correlated with disease activity than serum anti-DNA antibodies (45), suggesting that they are intimately associated with SLE.

One of the most striking features of OA is its ability to stimulate tumor necrosis factor (TNF) (46) by mimicking the phosphorylation patterns of TNF/IL-1 (44). An overabundance of TNF causes receptors to be shed from cells (47). As with soluble IL-2 receptors, soluble TNF receptors retain the ability to bind TNF, so the net effect of a hyper-stimulation of TNF might be little or no functional TNF, because the cytokine is binding to shed receptors. If an OA-like toxin was present in SLE patients, able to stimulate TNF, it would be expected that the patients would exhibit dysfunctional, or possibly low, TNF. In fact, this has been reported (10).

One of the effects of chloroquine (which is used to treat SLE patients) is that it inhibits the production of TNF and IL-6 (48), although the mechanism remains elusive.

Mechanism of immune disruption caused by okadaic acid-like toxin

In addition to damaging ion-dependent organs such as kidney and heart, there is compelling evidence that the disruption of calcium (and other ions) can have a profound effect on the signalling pathways of lymphocytes. OA is a serine/threonine phosphatase inhibitor (49), and it is capable of mimicking the protein phosphorylation patterns of IL-1 and TNF through the induction of immediate early response genes *c-jun*, *c-fos*, *egr-1* (44,50,51) and the transcription factors NF κ -B and AP-1 (52,53). While OA may

have the ability to induce transcription of genes which phosphorylate proteins necessary for lymphocyte signalling, it may block crucial secondary signals in the autocrine growth pathway.

The signalling pathways of lymphocytes are protein phosphorylation and dephosphorylation-dependent (for review see 54). In T lymphocytes, within seconds of T-cell receptor (TCR)/CD3 binding, phospholipase C-catalyzed hydrolysis of cellular membrane occurs which causes changes in cytoplasmic ion concentrations. Influx of extracellular Ca activates protein kinase C (PKC) and the regulatory protein calmodulin which catalyze phosphorylation of serine, threonine, and tyrosine residues. Deactivation of the proteins by phosphatases is crucial for secondary signalling processes which eventually activate genes in the nucleus, resulting in growth, proliferation and cytotoxicity (55,66).

IL-2-induced signalling of T-cells occurs through the Ca-mediated phosphorylation of protein tyrosine kinase (PTK) p56 on serine-threonine residues (57). OA inhibits serine-threonine phosphatases 1 (PP1) and 2A (PP2A) (49). It also inhibits influx of extracellular Ca and mobilization of Ca from intracellular stores, and inhibits the opening of Ca channels in cell membranes in various blood cells (29,58,59). By disrupting Ca concentrations, OA can potentially interrupt the IL-2 signalling process by preventing dephosphorylation.

Inhibition of CD45-mediated dephosphorylation of p56^{lck} by okadaic acid-like toxin results in defective T-cell activity

SLE patients have specific T-cell abnormalities, including decreased CD45 T-cells (2,4), decreased CD45RA naive suppressor/inducer subsets (2,3), and defective CD2 and CD3 signalling (3,6,7). The actions of OA on signalling processes could account for these defects, as follows.

CD45 is a tyrosine phosphatase transmembrane receptor (60,61) which is necessary for T-lymphocyte proliferation (55). CD45 modulates signals induced by various receptors such as CD4, CD8 and CD3 (62,63). It is also necessary for T-cell proliferation signals through CD2, as well as cytotoxicity of T and NK cells after exposure to target cells (55,64,65).

The CD4, CD8, CD3, CD2, and NK cell defects in SLE patients may relate to defective CD45 signal transduction through the IL-2-mediated PTK p56 pathway. IL-2 causes the phosphorylation of p56^{lck} on serine/threonine residues (66). The phosphorylation is Ca²⁺/calmodulin-dependent (57). CD45 dephosphorylates p56^{lck} (62). CD45 is crucial for CD4-mediated p56^{lck} activity (67) which suggests that CD45 dephospho-

phorylation of p56^{lck} is necessary for CD4-induced activity (i.e. the production of IL-2 necessary for the growth of CD4 lymphocytes and the cytotoxicity of CD8 lymphocytes).

Bajpai and Brahmī have demonstrated that OA reduces the cytotoxicity of cytolytic T lymphocytes (CTLs) and that the reduction involves CD45 and serine/threonine phosphatases (65). OA mimics the inactivation of cytolytic T lymphocytes when they are stimulated by target cells. IL-2 reactivates the T cells. SLE patients have deficient cytolytic T-cell activity which is reversible with IL-2 (68,69). This is consistent with the presence of a toxin like OA which can disrupt serine/threonine de-phosphorylation-dependent IL-2 signalling.

The cytotoxicity-inducing effect of IL-2 on T lymphocytes is dependent on p56^{lck} (70). P56^{lck} is also crucial for the maturation, selection, and activation of T cells (for review see 54). Although no studies have been done, to this author's knowledge, it is likely that p56^{lck} phosphorylation is impaired in SLE patients, and that this unique disruption accounts for IL-2-mediated CD4 and CD8 defects in these patients. A recent study found low levels of p56^{lck} in asymptomatic human immunodeficiency virus (HIV)-positive patients with impaired CD4 T-cell proliferation (70), as in SLE patients (8). Defective CD3 activation was noted in these patients as well. This could be the result of a toxin-induced impairment of p56^{lck} dephosphorylation.

The conclusion can be drawn from this data that the OA-like toxin in SLE patients, which is a phosphatase inhibitor, is inhibiting the CD45 dephosphorylation of p56, thus impairing CD4/IL-2-mediated signals.

Further compelling evidence that SLE patients have a CD45-mediated p56^{lck} signalling defect involving an OA-like toxin, which perturbs Ca and inhibits serine/threonine phosphatase, is that SLE patients exhibit decreased CD45RA naive T cells (2,4) and increased CD45RO (71). Nagelkerken and Huijbregts have recently demonstrated an association in mice between CD4 CD45RA and CD45RO phenotypes, and calcium (72). In aging mice, the CD45RA naive phenotype decreases in favor of the CD45RO memory phenotype *because of an inability of CD45 cells to increase and mobilize intracellular calcium* which is crucial for the naive phenotype.

Okadaic acid-like toxin in relation to interleukin 2 dysregulation in systemic lupus erythematosus

SLE patients are reported to have elevated IL-2 (11), decreased secretion (7), elevated soluble IL-2 receptor (sIL-2r) (73,74), and decreased IL-2 receptor (IL-2r) (71). The IL-2r has an α and β chain. Association

of p56^{lck} with the IL-2r β chain is crucial for IL-2-induced activation of p56, specifically serine-rich sequences within the β chain (75,76). SLE patients have decreased expression of IL-2 β (77).

Elevated sIL-2r may relate to the regulation of CD4/CD8 phenotypes in the following way:

When IL-2 binds its receptor, p56 phosphorylates the IL-2r β chain (75). Phosphorylation of p56^{lck} results in an apparent molecular mass change from 56-kD to 60-kD (66), and this correlates with phosphorylation of a serine 59 residue. Nakamura et al have reported that in human T cell lymphotropic virus (HTLV)-transformed T-cell lines, the Ick gene contains two separate promoters which direct the transcription of different products. In IL-2-dependent T-cell lines, only the upstream promoter is expressed, whereas in IL-2-independent lines, both upstream and downstream promoters are expressed (78). This author hypothesizes that the 'extra' transcript may be a down-regulating factor.

Activated T lymphocytes release soluble IL-2 receptors (sIL-2rs), which are capable of binding IL-2. The sIL-2rs may act to down-regulate IL-2-mediated activation of T cells by binding to IL-2 (79). Although sIL-2rs bind IL-2, they cannot act as cell-surface receptors, and they have a lesser molecular weight (45–50 kD versus 55 kD). This author suggests that there is differential IL-2 regulation of IL-2r versus sIL-2r which is mediated through the serine 59 residue of p56^{lck}.

One way OA, or an OA-like toxin, could cause the elevated sIL-2r in SLE patients would be through an ability to preferentially down-regulate IL-2r in favor of sIL-2r through binding with the IL-2r or affecting serine phosphorylation. Richard et al have demonstrated that OA upregulates initial IL-2mRNA at the same time it down-regulates IL-2r α mRNA (80). This might suggest that OA preferentially causes the production of sIL-2r as opposed to IL-2r. This would have the effect of nullifying the IL-2-mediated processes of T cells. The ability of OA to affect signalling processes through serine/threonine phosphatase inhibition or ion disruption is a plausible explanation for both elevated sIL-2 receptors and sTNFRs in SLE patients.

Involvement of okadaic acid-like toxin in activating latent and persistent viruses

Herpesviruses are activated in active systemic lupus erythematosus patients

The symptoms of SLE, along with the waxing and waning nature of the disease, suggests reactivation

of a latent virus or other persistent infectious agent. Studies in SLE patients have demonstrated elevated titers of antibodies to various herpesviruses (81,82); however, no systematic study has ever been undertaken (to this author's knowledge) which would demonstrate whether or not activated herpesviruses are uniquely related to SLE.

Accordingly, this author undertook a preliminary study of ten SLE patients fulfilling the American College of Rheumatology criteria for SLE. The patients were selected from the clinical practice of a rheumatologist (Daniel J. Wallace MD), and all were assessed by the clinician to be 'active' SLE patients. A commercial lab (Specialty Laboratories, Santa Monica, CA, USA) assayed the patients' sera for the presence of antibodies to six of the seven known human herpesviruses, plus rubella. Four patients were also tested for parvovirus B19, and one for measles. Patients exhibited significant titers of antibodies over controls. This author believes that the titers represent a genuine reactivation of latent and persistent viruses, and thus, some trans-acting or repression-inhibiting factor is present.

Herpesviruses are potentially activated in systemic lupus erythematosus patients vis-à-vis a toxic molecule which interferes with repressive mechanisms

If herpesviruses are indeed activated in SLE patients, the question arises as to where and by what means. In herpes simplex virus (HSV)-1 it has been demonstrated that the expression of viral genes leading to activation occurs in a cascade fashion, from immediate early (IE) to delayed early (DE) to late (L) (83). The cascade in HSV-1 is thought to be a prototype for all herpesviruses. The expression of IE genes is provoked by a viral protein, VP16 (also called Vmw65 and α -TIF) (84).

VP16 is a viral protein found in the tegument which is delivered into the host cell upon infection. Once there, it complexes with a host protein 'host cell factor' (HCF) (85). After VP16 binds HCF, a host-encoded octamer motif-binding factor (oct-1) then binds them both, causing a stable complex to form (86). VP16 and oct 1 bind the *cis*-regulatory sequence TAATGARAT of the IE gene promoter, and activate transcription (87).

It has been shown that VP16 peptides *inhibit* the formation of the HCF/VP16 complex (88), and that they interact with HCF (89). Wu et al have identified three amino acids on the peptides crucial for inhibitory activity, and suggest that they are critical contact points between VP16 and HCF (90). The predicted motif of HCF is predominantly threonine, serine and

proline residues and contains eight repeats of a novel 26 amino acid motif, each of which contain four conserved threonine positions (85).

The inhibitory activity of VP16 may occur through the phosphorylation and dephosphorylation of proteins located on HCF and VP16. It is suggested that, like T-cells, the inhibitory signalling involves serine/threonine phosphatases. Consistent with this theory, it has been demonstrated that the functional equivalent of VP16 in adenovirus – the E1A protein – has *repression* activity which is serine phosphorylation-dependent (91,92). The repressive activity is dependent on the association with another protein which is a product of the retinoblastoma gene (pRb) (93). It is suggested that this is reminiscent of VP16 associating with HCF to create repressive activity. In one type of experiment, OA blocked the phosphorylation of pRb (94) which suggests that it can also block the phosphorylation of VP16 which is necessary for repression signals in herpesviruses. It is possible that the OA-like toxin in SLE patients blocks the herpesvirus inhibitory process by inhibiting serine/ threonine phosphatase-dependent repression, thus leading to reactivation of these viruses.

In addition to activation through the inhibition of a common repressor gene, activation of herpesviruses in SLE patients might also be a function of the trans-activation capacities between the viruses. It has been demonstrated that herpesviruses have transactivating potential (95-97).

It is also notable that, in the experiments of Wu et al, the formation of the VP16 complex was inhibited by Mg^{2+} , suggesting that biochemical VP16 trans-activation processes are voltage channel-dependent. This is another potential site of OA disruption of normal activating/latent processes in herpesviruses which are ion-dependent. Either one of these disruptions (repression function or perturbation of ion-dependent activation) could account for the re-activation of herpes and other viruses in SLE patients.

Discussion

It has been demonstrated in a preliminary experiment that an OA-like toxin is present in the sera of SLE patients. Whatever the etiology of the toxin, the pertinent concept is that the immune dysregulation characteristic of SLE may be toxin-related. Data from published studies suggests that a toxin in SLE patients could perturb crucial immune-cell signalling through serine/threonine phosphatase inhibition and ion disruption could cause the organ damage associated with SLE.

Additional evidence has been presented for the

re-activation of latent and persistent viruses in SLE patients. An hypothesis has been suggested where an OA-like toxin transactivates this class of viruses through inhibition of the cell-virus-mediated repressive mechanism. The unregulated transcription of viral proteins somehow may provoke anti-DNA antibodies, given that these types of viruses are integrated into host DNA. The proliferation of antibodies to viral proteins in SLE is probably a result of partial transcription of the viral genome.

A variation of this hypothesis may be applicable to other diseases, including chronic fatigue immune dysfunction syndrome (CFIDS), and AIDS.

In order to make progress in the treatment of SLE, researchers must abandon the concept of antibody formation in SLE as an 'epi-phenomenon', and look towards more pragmatic concepts elucidated by research into the molecular basis of immunity. No evidence from recent immunology research suggests that antibody formation is either spontaneous or genetically controlled, yet this has been the prevailing explanation for the extreme disruption of immunity in SLE patients.

The origin of the toxin is the subject of ongoing research by this author. Viruses, bacteria and algae are all known to be capable of producing toxins which disrupt crucial immune and organ processes consistent with what is observed in SLE and other disease states (98–100). A strange organism, possibly associated with algae, has been linked to severe diarrhea in travellers and AIDS patients (101). Algae are hosts for algae viruses (102).

Achievements in immunology and virology which have occurred over the last decade will be valuable tools in identifying the cause of SLE and other immune-disrupting diseases.

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