Granulomas in Murine Schistosomiasis Mansoni Have a Somatostatin Immunoregulatory Circuit

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The role of somatostatin (SRIF) in controlling the granulomatous inflammatory response to infection with the parasite *Schistosoma mansoni* was explored in mice. The murine granulomas contain SRIF-14. Immunoreactive SRIF and preproSRIF localize in the cytoplasmic granules of macrophages within the granulomas. The granulomas contain mRNA for preproSRIF and are not innervated. The production of SRIF by the inflammatory cells appears to be inducible. The granulomas contain mRNA for the SRIF receptors sst_2A and sst_2B, which are expressed mainly on $CD4^+$ T lymphocytes and bind SRIF-14 with high affinity. Antigens from the schistosome eggs stimulate granuloma T lymphocytes to produce cytokines. Interferon-γ (IFN-γ) is one such cytokine made by $CD4^+$ T lymphocytes. SRIF-14 suppresses antigen-induced IFN-γ production from granuloma cells, and this effect is blocked by anti-sst_2 antibody. SRIF was shown to inhibit IFN-γ–induced immunoglobulin G2a (IgG2a) synthesis in murine schistosomiasis. SRIF also blocks substance P (SP)-stimulated IFN-γ and IgG2a secretion. Schistosome-infected animals treated with the SRIF analog octreotide form smaller granulomas that secrete substantially less IFN-γ and IgG2a. Unpublished observations suggest that SRIF does not modulate schistosome egg antigen- or concanavalin A–stimulated granuloma lymphocyte proliferation in murine schistosomiasis. In conclusion, SRIF may be an important factor in the control of the granulomatous inflammatory response in murine schistosomiasis.

GRANULOMAS are chronic, focal inflammations composed of macrophages and other immune cell types. The immune system frequently mounts a granulomatous-type response when it encounters factors like microorganisms and foreign substances that endure in host tissue. Granulomas usually protect the host by surrounding and preventing the spread of these harmful substances, which are resistant to destruction. A single granuloma can last for years. Granulomas are complex inflammations using many immunoeffector mechanisms controlled by various immunoregulatory circuits.[1]

Schistosomiasis mansoni is a common parasitic disease in which flukes exist in the portal vein of the host. The disease is indigenous to tropical and subtropical regions of the world. The parasitic flukes (schistosomes) produce eggs that settle in the liver and intestines. The ova survive for weeks, releasing antigens that incite a chronic granulomatous inflammation. Schistosomes can infect mice and incite granulomas similar to those produced in humans. In the laboratory, it is easy to infect and house colonies of mice harboring *Schistosoma mansoni.* This allows investigation of the cell-cell interactions and soluble factors governing the induction and maintenance of the resulting granulomatous response. Moreover, it is of considerable interest that murine schistosomiasis mansoni has a somatostatin (SRIF) immunoregulatory circuit operating within the liver and intestinal granulomas.[1,2]

SRIF AND IMMUNOREGULATION

There are two major forms of SRIF (SRIF-14 and SRIF-28), both deriving from one precursor molecule. SRIF-28 contains 28 amino acids, including the entire 14 amino acid sequence of SRIF-14. Endocrine and paracrine cells at mucosal surfaces, as well as nerves, can make SRIF.

In murine schistosomiasis mansoni, the granulomas contain SRIF-14.[3] The SRIF is authentic as shown by high-performance liquid chromatography (HPLC) and radioimmunoassay. There is no detectable SRIF-28. It is likely that granuloma macrophages make SRIF, since both immunoreactive SRIF and preproSRIF localize in the cytoplasmic granules of these cells. Also, we have two macrophage cell lines in our laboratory that express preproSRIF mRNA. Moreover, the granulomas contain mRNA for preproSRIF, which has been confirmed by nucleotide sequencing (manuscript in preparation). The granulomas have no nerves.[4] This suggests that the inflammatory cells are the only source of SRIF within the granulomas. The liver has little innervation, whereas the intestines are rich in nerves that produce SRIF. Granulomas destroy and displace nerves.[4] Yet the intestinal granulomas, unlike those of the liver, form near nerves capable of SRIF release. Thus, it is still conceivable that neuronal SRIF contributes to immunoregulation of granulomatous inflammation at mucosal surfaces.

Schistosome granuloma macrophages cultured in vitro secrete immunoreactive SRIF.[3] Macrophages stimulated with secretagogues release more. There is also preproSRIF mRNA in the spleen of infected mice, but even reverse-transcriptase polymerase chain reaction (PCR) does not yield preproSRIF cDNA product from spleen cells of uninfected animals (manuscript in preparation). These cell culture and PCR experiments suggest that the production of SRIF by inflammatory cells is inducible.

Lymphocytes express SRIF receptors. There are at least five distinct SRIF receptors, designated $sst_{1\text{-}5}$, expressed in humans and rodents. Also, alternate splicing can produce two forms of sst_2 (sst_2A and sst_2B). In murine schistosomiasis, the granulomas and spleens contain authentic sst_2A and sst_2B mRNA, but do not appear to express other SRIF receptors.[5] In the granuloma, sst_2 is expressed predominantly, if not exclusively, on $CD4^+$ T lymphocytes.[5,6] The receptor engages SRIF-14 with high affinity. SRIF-28 does

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not bind. Murine CD4+ T-cell lines, derived from the granuloma, also express sst_2.[5] Previous studies suggest that the human Jurkat leukemic T-cell line, the human immunoglobulin E (IgE) producing myeloma cell line U266, the murine plasmacytoma MOPC-315, and others, all express SRIF receptors.[7-9] Also, using a direct binding assay and flow cytometry, it has been reported that large numbers of murine Peyer's patch and splenic lymphocytes bind SRIF.[10] Germinal centers of human gut-associated lymphoid tissue may also bear SRIF receptors as assessed by autoradiography of tissue sections.[11] Moreover, radiolabeled SRIF analog scintigraphy visualizes human granulomas and lymphomas in vivo.[12,13] Thus, the human immune system may frequently display SRIF receptors prominently.

Some investigators have reported that both isoforms of sst_2 are negatively coupled to adenylyl cyclase in sst_2-transfected cell clones.[14-16] Although SRIF can modulate immune function, we have not seen alterations in granuloma or splenic cyclic adenosine monophosphate (cAMP) on SRIF exposure.[17]

Granuloma T cells make lymphokines that are important for controlling the granulomatous response. Soluble antigens from the schistosome eggs stimulate their production. Interferon-γ (IFN-γ) is one such cytokine made by CD4+ T cells.

SRIF-14 suppresses antigen-induced IFN-γ production from granuloma cells or splenocytes cultured in vitro.[6] Also, antibody that specifically blocks sst_2 completely prohibits this phenomenon (manuscript in preparation). Thus, SRIF appears to act directly through the sst_2-bearing T cell that makes IFN-γ. SRIF does not modulate interleukin-4 (IL-4), IL-5, IL-6, or IL-10 secretion in short-term cultures maintained under similar conditions.

IFN-γ possesses antiviral activity and exerts pleiotropic immunomodulatory activities, including activation of macrophages and natural-killer (NK) cells, and enhancement of major histocompatibility complex (MHC) class I and II expression. Also, IFN-γ promotes the switching of murine B-cell immunoglobulin production to the IgG2a isotype. Moreover, the secretion of IgG2a in murine schistosomiasis is dependent on IFN-γ. We showed that through its effect on IFN-γ production, SRIF markedly inhibited IgG2a synthesis in murine schistosomiasis.[18] The effect was on both polyclonal, as well as schistosome egg antigen-specific IgG2a-producing B cells. Substance P (SP) is another neurokine made in the granuloma that, contrary to SRIF, enhances IFN-γ production.[19] SRIF can completely antagonise SP stimulation of IFN-γ and IgG2a secretion. It does not alter IgG1 or IgM secretion. Yet others have reported, using healthy animals from a different murine species, that SRIF suppresses IgA and IgM secretion in long-term in vitro lymphocyte cultures.[20] Also, tonsillar lymphocytes from atopic individuals cultured in vitro for 14 days produce less IgE spontaneously in the presence of SRIF, but normal amounts of other antibody subtypes.[21] The latter effect required T cells and monocytes.

Octreotide administered parenterally can modulate inflammation. Octreotide is a biologically stable SRIF receptor agonist that binds granuloma SRIF receptors with an affinity higher than that of native SRIF-14. As predicted from our in vitro experiments, schistosome-infected animals treated with octreotide form smaller granulomas that secrete substantially less IFN-γ and IgG2a.[18] Yet, the granulomas continue making the IFN-γ–independent antibodies IgG1 and IgM. There are now several additional reports suggesting that somatostatin analogs can suppress an inflammatory reaction in vivo in rodents.[22,23]

SRIF may affect the capacity of lymphocytes to proliferate in response to stimuli. SRIF can inhibit proliferation of phytohemagglutinin-stimulated human blood T-cell and Molt-4 lymphoblasts in vitro.[24] After intravenous infusion of SRIF, peripheral blood lymphocytes from patients with bleeding duodenal ulcers show diminished responsiveness to mitogens and enhanced spontaneous IL-2 receptor expression.[25] Also, SRIF may decrease murine lymphocyte proliferation both in the presence[20,26] or absence[27] of mitogens. However, in murine schistosomiasis, SRIF does not appear to modulate the proliferation of granuloma or splenic lymphocyte in response to schistosome egg antigens or concanavalin A during short-term cultures (unpublished observation). In the human lamina propria, SRIF may inhibit IL-2 receptor expression and immunoglobulin production, but effects lymphocyte proliferation poorly.[28]

Furthermore, SRIF may influence other aspects of an immune response. As with SP, SRIF is a secretagogue for human, mouse, and rat mast cell products, but only when used in high concentration.[29-32] Thus, this phenomenon may not be physiological. Also, there are individual reports suggesting that SRIF modulates basophil secretion,[33] leukocyte migration inhibition factor production,[34] NK-cell activity,[35] the graft-versus-host reaction,[36] and lymphokine-activated killer-cell activity.[37]

CONCLUSIONS

We have shown that granuloma macrophages make SRIF-14. Also, the granulomas express SRIF receptors of the sst_2 subtype. They are present on the granuloma CD4+ T cells. Through these receptors, SRIF regulates IFN-γ secretion from the granuloma CD4+ T cells and other cellular functions such as IgG2a expression. Modulating SRIF action within the granulomas in vivo substantially alters aspects of the inflammatory response, attesting to the importance of this neurokine within the inflammation.[5]

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