ABSTRACT

Recurrent aphthous stomatitis (RAS) is an ulcerative disease that most often occurs in otherwise healthy individuals and presents as a painful lesion of the buccal and labial mucosa and tongue. The prevalence of RAS in the general population ranges between 5 and 25%. The modifying factors that aggravate the response in RAS include: various food allergies, vitamin and mineral deficiencies, hormonal and gastrointestinal disorders (e.g., celiac disease, Crohn’s disease, ulcerative colitis), some viral, bacterial and fungal infections, mechanical injuries, genetics, hereditary factors, psychosomatic disorders and stress. There is correlation between inheritance of specific gene polymorphisms related to proinflammatory cytokines & development of RAS. Thus, RAS is considered to be multifactorial disease.
**Immunohistopathogenesis of RAS**

In the previous literature there exists relationship between several immune-mediated reactions and development of RAS. These immune reactions includes (1) cytotoxicity of T lymphocytes to oral epithelium, (2) antibody-dependent cell-mediated cytotoxicity, and (3) defects in lymphocytic populations. The immune reactions cause damage due to deposition of immune complexes within the oral epithelium.

There are studies which had shown an association between RAS severity and abnormal proportions of CD4+ and CD 8+ cells, with altering the CD4+:CD8+ ratio, and elevating levels of interleukin 2 (IL-2), interferon gamma (IFN), tumor necrosing factor-alpha (TNF-α) and mRNA in RAS lesions. There is no change in the levels of serum immunoglobulins and natural killer cells in RAS patients. Thus the focus is still on a dysregulated, local, cell-mediated immune response which is responsible for accumulation of subsets of T cells, mostly CD8+ cells. There is increase reactivity seen in relation to local immune response which causes eventual tissue breakdown that manifests as RAS.

The key principle molecules that are involved in...
understanding the immunohistopathogenesis of RAS are:

1. Th1 type Cytokines
2. Th2 type Cytokines
3. Mast cells

Th1 type cytokines, which include: IL-2, IL-12, IFN-γ and TNF-α, determine the predisposition towards autoimmunization, induce the cellular type response and stimulate the secretion of IgG. Th2 type cytokines, including: IL-4, IL-5, IL-10 and IL-13, manifest anti-inflammatory properties, stimulate the humoral immune response and the secretion of IgE.

Strong anti-inflammatory effect is also contributed to another cytokine called transforming growth factor (TGF)-β, secreted mainly by the lymphocytes. Both types of the immune response humoral and cellular are dysregulated in patients with RAS, with manifestation of neutrophils reactivation and hypereactivity, increased levels of the complement component, increased number of NK cells and B lymphocytes, altered ratio of CD4 & CD8 ratio and increased number of CD and T cell receptor (TCR)CD cells. There is significant increased secretion of Th1 cytokines in RAS patients. There is also increased production of IL-2, IFN-γ and TNF-α by the peripheral blood mononuclear cells in the acute phase of the disease and in the remission. There is also increased number of T lymphocytes produced pro-inflammatory cytokines (IL-2, IL-12 and IFN-γ) and the decreased number of IL-10 producing cells in the blood of the RAS patients.

According to histology, three stages are noted in the development of RAS:

1. Preulcerative stage
2. Ulcerative stage
3. Healing stage.

Preulcerative stage is characterized by lymphocytic infiltration in the epithelium focal vacuolation and is followed by degeneration of the suprabasal epithelial cells accompanied by a mononuclear, mainly lymphocytic infiltrate in the lamina propria. This stage demonstrates subepithelial inflammatory mononuclear cells with abundant mast cells. There is evidence of connective tissue edema, and lining of the margins with neutrophils. Damage to the epithelium usually begins in the basal layer and progresses through the superficial layers, leading eventually to ulceration and surface exudate. In this stage number of CD4+ cell population is increased.

Ulcerative stage shows increased infiltration of the tissues, particularly the epithelium, by mononuclear cells and accompanied by more extensive edema and degeneration of the epithelium progressing to frank ulceration with a fibrinous membrane covering the ulcer. The presence of extravasated erythrocytes around the ulcer margin, subepithelial extravascular neutrophils, numerous macrophages loaded with phagolysosomes, and the nonspecific binding of stratum spinosum cells to immunoglobulins and complements may be a result of vascular leakage and passive diffusion of serum proteins. These findings suggest that pathogenesis of RAS may be mediated by immune complex vasculitis.

There are more numerous CD8+ cells during the ulcerative state of the ulcer. Healing stage is characterized by regeneration of the epithelium. Immunohistochemical studies of RAS biopsy tissues have demonstrated numerous inflammatory cells with variable ratios of CD4+:CD8+ T lymphocytes depending on the ulcer duration. CD4+ cells were more numerous during the preulcerative and healing stages. Also there is decrease immune response of patients' lymphocytes to mitogens and alterations in the activity of natural killer cells in various stages of disease.

The infiltration of the epithelium by T lymphocytes is likely to be in response to some, as yet unidentified, keratinocyte-associated antigen. Keratinocyte death is thought to be mediated by the differentiation of cytotoxic T cells and involves the production of TNF-α by these and other leukocytes. TNF-α induces inflammation by its effect on endothelial cell adhesion molecule (ECAM-1) and neutrophil chemotaxis. Other cytokines, e.g. IL-1 and IL-2, may also play a role in the immunopathogenesis of RAS.

The steps involved in immunopathogenesis of RAS could be summarized as follow:
Antigenic keratinocyte stimulation

Activation of mitochondrial HSP (Heat shock protein)

Activates T-cell mediated immune response

Activation of cytotoxic CD8+ cell

Alteration of CD4:CD8 ratio

Antibody- dependent cellular cytotoxicity

Deposition of immune complex in epithelium

Increase IL-2, IFN, TNF-α

Inflammatory events

Increase ECAM-1, Neutrophil chemotaxis

Increase IL-1β, IL-2, IL-4, IL-5, IL-6, IL-12

Increase anti-inflammatory cytokines

Decreased TGF-β & IL-10

Imbalance between proinflammatory & anti inflammatory cytokines

RAS

Role of mast cell: Mast cell are mobile, bone marrow derived, typically containing 80-300 granules. MC degranulation is induced by various stimuli such as IgE receptors, Neuroropeptides (substance P), chemokines (RANTES) (regulated on activation, normal T cell expressed and secreted) & various other physical stimulus. Degranulation of MC releases pro-inflammatory mediators such as: TNF-α, chymase, tryptase, matrix metalloproteinases (MMPs), basic fibroblast growth factor (bFGF), heparin, histamine, various ILs (IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-16 and cytokine RANTES). Mediators secreted following activation of MC are: IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13 & IL-16, platelet activating factor (PAF), RANTES, macrophage inflammatory protein (MIF-α) & arachidonic acid metabolites, prostaglandin & leukotriene C4 (LTC4).

Role of Mast cell released cytokines:

IL-3 – induce basophil recruitment & activation
IL-5 – eosinophil recruitment & activation
IL-13 – induction of IgE synthesis of B–cell
Mast cells can promote inflammation in the absence of IgE mediated activation & likely infiltrate inflammatory events under many circumstances.

Degranulation of mast cell

Cytokines

Release protease & Activates MMP-9

Tissue degradation

Damage to basal cell

Ulcera
Mast cells also play potential role in the development of RAS by their ability of secretion of various inflammatory mediators via degranulation.\textsuperscript{25} The mast cell count in the first 2 days does not differ from that of the normal buccal mucosa, but there is an approximately 50% reduction in mast cell count in lesions of more than 48 hour duration. In contrast, increased numbers of mast cells are noted in all three types of RAS (minor, major and herpetiform), particularly with toluidine blue stain. In RAS mast cell numbers has been found more as compared to healthy oral mucosa which may be due to its secretion of inflammatory mediators.\textsuperscript{2,18}

**RECURRENCE OF RAS**

The various predisposing and precipitating factors in the development of RAS are mainly genetic associated triggering factors, imbalance in the immune system, malnutrition, hormonal upset, type A personality, infectious agents and stress. These factors are mainly responsible for recurrent episodes of RAS. The size and duration of ulcer depends on intensity and duration of the trigger factor in a susceptible individuals. Stress is directly related to the recurrence of RAS. There is elevated levels of salivary cortisol which is thought to be a biomarker of stress. Thus recurrent episodes of RAS may be due to various etiologic factors and stress experience by an individual.\textsuperscript{31,32} Stress causes alteration in immune system and also affects distribution, proliferation and activity of lymphocytes, and natural killer cells and production of cytokines. Also high prevalence of RAS is seen in low socio economical class which could be associated with poor oral hygiene, malnutrition iron and multivitamin deficiencies, resulting in decrease immunity and increase susceptibility towards recurrent episodes of RAS.\textsuperscript{33}

**MANAGEMENT OF RAS**\textsuperscript{34,35,36,37}

In the management of RAS, the size, frequency, pain, site, and recurrence bouts of ulcers are important. Basically treatment protocol for RAS falls under categories like:

1. Symptomatic therapy with topical applications of various pharmacological preparations such as: Orabase alone or mixed with topical anaesthetic such as benzocaine to minimize patient discomfort.
2. Also depending on intensity of pain, NSAIDs, or diclofenac can be given orally or locally.
3. In case of large major aphae topical and oral formulations may not give complete healing and comfort, there we can use intralesional steroids to fasten the healing. Along with steroids, topical antibiotics and tetracycline mouth rinses also promotes better healing and comfort to patient. A short course of systemic corticosteroids such as prednisone can be used to treat a severe episode of major RAS.
4. Also as a preventive measures for recurrent episodes of RAS antioxidant supplement can be beneficial. As it is well known fact that antioxidant combat the free radicals and are effective against reactive oxygen species (ROS). Stress causes increased release of free radicals and ROS, thus antioxidant supple- ment can prove beneficial to prevent recurrence of RAS. Also there are newer targeted therapy against TNF-\(\alpha\) to treat RAS in a susceptible individuals having genetic predisposition. Thus inhibitors of TNF-\(\alpha\) can help to prevent recurrent bouts of RAS.\textsuperscript{38,39}

**DISCUSSION**

Immune dysregulation is now a generally accepted cause of RAS. In our review we have touched upon the various immune factors responsible for the etiopathogenesis of RAS. Extensive research in the advances in the understanding of immunopathogenesis has led to the formation of targeted therapy against these agents. The Th 1 cytokine producing cells are upregulated in RAS. The study carried out on the Behcet’s disease and RAS showed a raised population of gdT cells and CD69.\textsuperscript{40} Th -2 cells
are CD4+ effector cells that produce IL-2 and TNF-a. Amongst the various cytokines implicated, role of TNF-a is very prominent. Newer studies are showing role of TNF-a inhibitors in RAS to be a very effective mode of therapy. TNF-a can also be used as a screening agent or indicator for the susceptibility of RAS. Serum levels of Ig G2 are also shown to reduced in RAS. Thus, a complete immuno-profile of the susceptible individuals can give us better means of combating this chronic illness. Oral aphthous ulcerations develop as a result of loss of epithelial barrier function and that nuclear factor kappa beta signaling pathway seems to be involved in this type of injury.

CONCLUSION

Considering the etiopathogenesis of RAS there is increased immune response due to proinflammatory cytokines towards specific antigens which is responsible for the development of aphthous erosions and ulcers. To summarise the immunological aspects of RAS, the important factors are lymphocytotoxicity, antibody-dependent cell-mediated cytotoxicity and defects in the lymphocyte T cell population. Cytokines such as IL-2 & IL-10 & depressors of natural killer cells are also believed to be involved in the development of RAS. The immunohistopathogenesis of RAS is strongly related to cell-mediated immunity & TNF-α. Even though the exact mechanism is not known, the formation of RAS probably involves cell-mediated immune response involving the T-cells, macrophages, mast cells and TNF-α production by these cells. TNF-α subsequently can induce inflammatory change due to its effects on endothelial cell adhesion and neutrophil chemotaxis. Underlying mechanisms relating to pathogenesis need to be explored further in order to establish the treatment protocol.

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