Salivary Gland Involvement in Graft-versus-Host Disease: the Underlying Mechanism and Implicated Treatment

Raphael M. Nagler MD DMD PhD[1] and Arnon Nagler MD MSc[2]

[1] Department of Oral and Maxillofacial Surgery, Oral Biochemistry Laboratory and Salivary Clinic, Rambam Medical Center, Haifa, Israel
Affiliated to Technion Faculty of Medicine, Haifa, Israel
[2] Department of Bone Marrow Transplantation, Sheba Medical Center, Tel Hashomer, Israel
Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

Key words: graft-versus-host disease, salivary gland, sialochemistry, sialometry, pilocarpine

Abstract

Patients with graft-versus-host disease suffer from xerostomia, oral infections and mucosal pathologies. The continuous increase in the number of patients treated with bone marrow transplants worldwide, combined with improved survival statistics result in a concomitant increase in the number of GVHD patients. The pathogenesis of GVHD is based on donor graft T lymphocytes that recognize antigenic disparities between donor and recipient, and on the disregulation of a broad panel of cytokines. Consequently, various tissues and organs, including the mucosa of the oral and gastrointestinal tract, are damaged via cytotoxicity caused by infiltrating T cells. Since the salivary glands are a known major target of GVHD and their secretions significantly contribute to preserving mucosal integrity, this mucosal insult is further enhanced by the reduced quantity and altered quality of saliva. GVHD occurs in 40–70% of patients treated by bone marrow and peripheral blood stem cell transplantation. Limited studies suggest that a large percentage of GVHD patients are affected and that the induced salivary dysfunction occurs rapidly following transplantation, affecting both major and minor salivary glands and reflecting the severity of the disease. Moreover, profound sialochemical alterations may be diagnostic of GVHD. An additional reason for the vast amount of research is that GVHD, as an autoimmune-like disease, seems to be an appropriate model for studying a much more prevalent, well-known and studied autoimmune disease involving salivary glands, namely, Sjögren's syndrome. The present review describes the GVHD-related sialometric and sialochemical data available in the literature for both major and minor salivary glands in both human and rodent models, and discusses a possible mechanism.

IMAJ 2004;6:167–172

Chronic graft-versus-host disease is a complex clinical entity that occurs after bone marrow transplantation. The disease is mediated by autoreactive T lymphocytes that infiltrate various target organs and tissues, including the oral mucosa and the salivary glands. In recent years, we and others have demonstrated both in animal and human models that T lymphocyte infiltration into the major salivary glands is accompanied by a concomitant loss of function, as expressed by salivary flow rate reduction (xerostomia) under both resting and stimulated conditions, and is a principal cause of morbidity. With an increasing number of unrelated and mismatched allo-stem cell transplantations and the change from bone marrow to peripheral blood grafts, the number of patients with GVHD is increasing [1].

Chronic GVHD may develop in 25–45% of recipients undergoing alloSCT from related matched siblings and its frequency increases to 40–70% in patients receiving alloSCT from unrelated matched donors [2]. The incidence is significantly lower in patients receiving human umbilical cord blood grafts and in those receiving haploidentical CD34+ purified grafts [3,4]. Chronic GVHD may develop as an extension of acute GVHD (progressive onset), after resolution of acute GVHD (quiescent onset), or without proceeding acute GVHD (*de novo* onset). Chronic GVHD is categorized as either limited (localized skin and/or hepatic involvement) or extensive (diffuse skin and/or multiorgan involvement); the latter is associated with a worse prognosis [5]. The introduction of low intensity conditioning and non-myeloablative alloSCT does not result in a reduced frequency of GVHD; on the contrary, it seems that the frequency is even increasing [6]. GVHD usually develops more than 100 days after transplant, with a tendency for later occurrence in patients receiving peripheral blood stem cell grafts and those undergoing low intensity conditioning alloSCT [1,6].

Chronic GVHD typically resembles a connective tissue autoimmune-like immunologic disorder, characterized by lichenoid or sclerodermoid skin lesions accompanied by joint contractions similar to systemic sclerosis. The clinical and pathologic features resemble the overlapping of several collagen vascular diseases and immune disregulations with eosinophilia, circulating autoantibodies, hypergammaglobulinemia, and plasmacytosis of viscera and lymph nodes [5]. The hallmark skin changes of the disease include papulosquamous dermatitis, plaques, dysquamation, dispigmentation and vetiligo. Chronic GVHD has histopathologic features similar to systemic sclerosis, manifested predominantly by sclerosis

GVHD = graft-versus-host disease

SCT = stem cell transplantation

of the thickened reticular dermis due to increased collagen synthesis [7]. The histologic distinction between papillary and reticular dermis may not be apparent because of sclerosis. Cutaneous appendages become encased in collagen and tend to disappear. There is variable perivascular and interstitial inflammatory cell infiltrate, composed predominantly of lymphocytes and occasionally plasma cells [8]. Less frequent skin findings include poikiloderma, reticulated hyperpigmentation, alopecia, dystrophic nails, leukoderma bullae, discoid lupus erythematosus, and photosensitivity. Less than 20% of patients with untreated extensive GVHD survive, with Karnofsky performance scores of at least 70% [5]. The pathophysiologic mechanism involves autoreactive lymphocytes and cytokine disregulation. The current therapeutic options are limited [9–11].

Effects of GVHD on salivary glands in rodent models

The pathologic changes revealed in GVHD rodent models resemble the alterations observed in a Sjögren-like syndrome. In general, a direct correlation is seen between the degree of the GVHD (expressed by weight loss and spleen weight increase, etc.) and its accompanying salivary hypofunction and altered composition, as well as salivary gland histopathologic changes.

In a rat model of GVHD, it was shown that secretory duct cells of both parotid and submandibular glands express HLA class I antigens [12], while a mononuclear cell infiltration was detected around the ducts of salivary glands in a murine model of GVHD [13]. In an acute GVHD mouse model [14], a significant decrease (61%) in the secreted salivary volume ($P < 0.01$) was accompanied by a change in the normal salivary flow rate curve into a plateau-shaped curve with elongation of the lag phase. The salivary functional loss was also accompanied by massive lymphocyte infiltration, nearly total parenchymal destruction, and a significant increase in salivary potassium. Control experiments suggested that these changes were induced by the GVHD process and not by the chemo-radiotherapy regimen preceding the stem cell transplantation [14]. In another study using a chronic GVHD mouse model [15], a significant reduction of the secreted saliva volume was observed in mice with active GVHD as compared to healthy controls or in mice that had undergone a syngeneic stem cell transplantation and did not suffer from subsequent GVHD. Three weeks following GVHD induction there was a reduction of 45% in the parotid flow rate ($P < 0.05$), compared to the syngeneic control group. In addition, a flattening of the normal salivary flow rate and a prolongation of the lag phase (time between pilocarpine stimulation and saliva secretion) was observed. Sialochemical analysis showed a significant decrease in sodium levels (42.1 ± 1.9 vs. 61.4 ± 4.8 mmol/L, $P < 0.01$) and a significant increase in potassium levels (20.0 ± 1.2 vs. 15.1 ± 1.2 mmol/L, $P < 0.05$) in the saliva of the GVHD mice as compared to syngeneic mice. Histopathologic evaluation of the parotid glands revealed a moderate lymphocyte infiltration, parenchymal destruction and fibrosis with a relatively high level of inflammation preceding both hyposalivation and fibrosis.

Sialometric and sialochemical effects of GVHD in humans

As previously noted, the pathogenesis of GVHD is based on donor graft T lymphocytes, which recognize antigenic disparities between donor and recipient. Consequently, the mucosa of the oral and gastrointestinal tract is affected by the cytotoxicity caused by the infiltrating T cells [16]. Since the salivary glands are also a major target of GVHD, the mucosal insult is further aggravated by the reduced quantity and altered quality of the saliva, which ordinarily contributes significantly to the preservation of mucosal integrity [17]. The consequences of the GVHD-induced salivary injury include patient suffering and a reduction in other related salivary functions, such as anti-inflammatory activity, protection against mechanical and chemical epithelial injuries and against periodontal disease and caries, and its role in verbal communication, nutrition, soft tissue repair, etc. [18].

A recently published long-term study reported that salivary gland involvement in GVHD is very rapid and severe, and that recovery does not occur within a year during which patients are monitored [19]. In this study, mean whole salivary flow rates were obtained at 0 time (prior to allogeneic peripheral blood stem cell transplantation) and at 2 and 12 months. The mean flow rate of the GVHD patients (n=12) prior to the allogeneic PBSCT was 0.59 ± 0.11 ml/min. The values of the flow rate for the allogeneic PBSCT disease-free individuals (n=6) and healthy controls (n=8) were similar, 0.42 ± 0.15 ml/min and 0.51 ± 0.13 ml/min respectively. In contrast to the control groups, in which the mean flow rates were not significantly altered during the year of follow-up, the flow rates in the GVHD group decreased gradually and were reduced by 39% and 70% at 2 and 12 months respectively ($P < 0.01$). Recognition of the salivary glands as a very sensitive target for GVHD is not surprising in light of the study published by Nakhleh et al. in 1989 [20]. That study showed that in 50% of the GVHD patients in whom there was minimal or no oral mucosal involvement (a well-established major target of GVHD), the salivary glands exhibited definite GVHD involvement. Reduction in whole saliva flow rate in GVHD patients has also been reported in other studies, as were various salivary biochemical and immunologic compositional alterations [21,22]. In conclusion, the available reports reveal a mean reduction of 55–90% in the salivary flow rate of GVHD patients [23,24].

A recently published sialochemical study [24] reported that GVHD patients had significantly higher salivary concentrations of sodium (395–454%, $P < 0.01$), magnesium (109–113%, $P < 0.05$), epidermal growth factor, total protein, albumin and immunoglobulin G (as high as 90%, $P < 0.05$) as compared to controls. These altered concentrations may compromise the functional capacity of saliva, which is directly related to normal salivary composition. As a result, the saliva may not be able to play its pivotal role in various functions, such as swallowing, masticating, controlling oral microorganisms, maintaining mucosal integrity and protecting against foreign proteins and infections, reducing the formation of period-

PBSCT = peripheral blood stem cell transplantation

ontal calculus and sustaining anticariogenic activity [18]. This reduced salivary capacity in controlling microorganisms in GVHD patients was demonstrated by Norhagen et al. [23], who found that patients with GVHD had less salivary IgM one year after bone marrow transplantation [23]. Furthermore, the altered salivary composition may act synergistically with the compromised salivary flow rate as previously discussed.

The reported three to fourfold increase in sodium concentration [24] is in agreement with two other fundamental studies in which Izutsu et al. [16] demonstrated an increase in sodium concentrations in the secretions of both minor salivary glands and whole saliva of GVHD patients, which could be accounted for by GVHD-induced and lymphocyte infiltration-mediated damage to the sodium reabsorbing salivary ductal system. A similar salivary sodium increase was demonstrated in Sjögren's syndrome patients as well, and the etiology suggested in this autoimmune disease was similarly based on the role played by infiltrating autoreactive lymphocytes. Concentrations of the three other electrolytes – potassium, calcium and phosphorus – were found to be similar in patients and controls [24]. That result is also supported by Izutsu et al. [16] who found similar resting salivary potassium concentrations in GVHD patients and controls.

With regard to anti-inflammatory activity and maintaining mucosal integrity in the oral cavity, it should be emphasized that salivary IgA and EGF are of major importance [25]. The significant salivary IgG increase in GVHD patients also concurs with the previously mentioned study of Izutsu et al. [16]. The significant elevation of EGF, total protein, albumin and IgG could result from direct GVHD-induced damage to the salivary parenchyma, as previously suggested. However, it might also be due to a transudation of serum components across the damaged and inflamed oral epithelium and gingiva, which are sites affected by the disease. Thus, it may be concluded that a multisite mechanism may be differentially responsible for the observed increase of various components which are either being secreted from the salivary glands as EGF or leak from the serum into the saliva as albumin. The salivary EGF is of special importance, and more so under resting conditions since resting salivary secretion is the dominant condition during most of the day and night; the dominant secreting salivary gland in this state is the submandibular gland [26]. In any event, a mere "concentrating effect" of reduced secreted volume due to a decrease in the watery component of the saliva (related to a specific insult to the muscarinic signal transduction pathway, for example) is excluded, since such a mechanism might explain an identical increase in EGF, total protein and immunoglobulins but not a differentiated one, as observed.

Immunologic, molecular and cytokine-implicated salivary alterations in GVHD

It is now known that molecules having a putative mechanistic role in GVHD are adhesion molecules such as up-regulators of the intracellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selection, which have been demonstrated in GVHD [27], indicating that the activation and injury of endothelium are commonly involved in the pathogenesis of GVHD. A cascade of signaling events directs and regulates the trafficking, homing and activating of T lymphocytes in GVHD. These adhesion receptors include selectins, integrins and adhesion molecules of the immunoglobulin superfamily. In addition, tissue-specific homing receptors direct the tissue-specific trafficking of T lymphocytes. It would be of interest to define and isolate the salivary-specific homing receptors that direct the cytotoxic T lymphocytes mediating the salivary GVHD-induced injury. Accordingly, treatment with antibodies against ICAM-1 or adhesion molecule VLA-4 blocked the adhesion-mediated tissue cell infiltration and improved liver injury in a GVHD mouse model [28]. Other adhesion molecules, such as CD31, CD49b and CD62L, have been identified as mono-histocompatibility antigens, which are proposed to be the key factors in GVHD following matched sibling transplantation [29].

Another new approach illustrating and emphasizing the role of cytokines in GVHD is cytokine gene polymorphism. Cytokine gene polymorphism is associated with functional differences in cytokine regulation. A correlation was found between the severity and frequency of GVHD and interferon-gamma, interleukins-6 and 10 and tumor necrosis factor-alpha gene polymorphism. Specifically patients homozygous for the IFNγ Intron allele 3 had more severe (Grade III-IV) acute GVHD. Similarly, patients possessing the IL-6 (-174) G allele had a trend toward higher grades of acute GVHD, and those homozygous for the IL-6 (-174) G allele were more likely to develop chronic GVHD [30].

Recently implicated in GVHD is the rather new cytokine, IL-18 [31]. Increased sera levels of IL-18 were found in patients who developed GVHD post-allogeneic PBSCT. IL-18 is a cytokine with wide-ranging biological functions including not only innate but also acquired immunity, eliciting both Th responses, particularly in collaboration with IL-12 and Th2 responses. IL-18 is involved in the development of cytotoxic T lymphocytes and natural killer cells, which may mediate the salivary injury observed in GVHD. Regarding the mechanism, in murine experiments it was demonstrated that recipient mice transplanted with H-2 disparate donor GLD/GLD spleen cells, which lack functional Fas lIgAnd (FasL), developed GVHD, but no elevation of IL-18 was observed, indicating that FasL mediates IL-18 release in GVHD. Furthermore, IL-18 elevation was found to be derived from host cells in a caspase-1-dependent manner [31].

Salivary mediated lesions in GVHD show histologic features of cell death with lymphocyte infiltration. It was recently demonstrated that preforin and granzyme B are involved in the process of apoptosis induced by cytotoxic T lymphocytes, which leads to epidermal injury in GVDH [32]. There may be a similar mechanism at work in the salivary injury in GVHD.

IL-13 is a new Th2 cytokine that was recently shown to suppress alloreactivity and to be of protective value in GVDH. Moreover, the

Ig = immunoglobulin
EGF = epidermal growth factor
ICAM = intracellular adhesion molecule
IFN = interferon-gamma
IL = interleukin

keratinocyte growth factor that has been demonstrated both in mice and humans as ameliorating GVHD may operate through IL-13 [31]. Keratinocyte growth factor and IL-13 may therefore be of therapeutic value in ameliorating GVHD-mediated salivary injuries. One of the mechanisms that may be critical to the attraction and recruitment of cytotoxic T cells to the salivary glands, thus mediating the GVHD-mediated injury, is the production of macrophage inflammatory protein 1 alpha, as it was recently shown that production of MIP-1α by donor T cells is important in the occurrence of GVHD and functions in a tissue-dependent fashion [33].

Another factor that may be implicated in salivary injury is heat shock protein-70. It was shown that increased levels of HSP-70 and antibodies reactive with HSP-70 parallel the onset and severity of GVHD. Moreover, deoxyspergualin, which ameliorates GVHD, has been shown to reduce HSP-70 levels, resulting in diminished serum levels of IL-2, IFNγ and TNFα, which have been implicated in GVHD-mediated end-organ injury [34].

A molecule that has been implicated in the tissue destruction of GVHD is nitric oxide. Increased levels of nitric oxide have been shown in GVHD, and blockage of nitric oxide production and pathways may have a therapeutic role. The onset of GVHD is accompanied by macrophage (M phi) priming, which results in expression of nitric oxide synthase and the production of nitric oxide in response to lipopolysaccharide. Continual exposure to IFNγ is required to maintain a primed state of M phi during GVHD. Indeed, increased IFNγ in RNA has been demonstrated in salivary gland tissue in mice with GVHD [35].

Possible treatment modalities

The general therapeutic options in GVHD have been rather limited until recently, consisting of immunosuppressive agents, mainly methylprednisolone and cyclosporine. Although such conventional therapy has achieved complete responses in approximately 50% of patients, the disease remains hardly controllable in most patients. Other therapeutic options that have been tried over the years include compounds like thalidomide, mycophenolate mofetil, tacrolimus, rapamycin, clofazimine, etretinate, hydroxycloroquine, ursodeoxycholic acid, penicillamine, cyclophenile, nedocromil sodium, as well as medical procedures such as induction of oral tolerance, total lymphoid irradiation, phototherapy (PUVA), and extracorporeal phototherapy [11].

The traditional means for inducing salivary hypofunction have also been rather limited, consisting of mouth rinses, artificial saliva and intra-oral anti-inflammatory gels, all offering only limited and transitory relief. However, in four different studies [24,26] performed in recent years, a new agent aimed at improving salivary function was successfully tested: pilocarpine hydrochloride (Salagen®, Megapharm, Israel). The salivary flow rate in six chronic GVHD patients, prior to and after 6 months of therapy with Salagen 30 mg/day, was recently assessed. It was found that such therapy resulted in a significant increase in the salivary flow rate of submandibular/sublingual, parotid and whole-saliva secretions. This increase was 224–289% ($P < 0.01$) under resting conditions and 134–247% ($P < 0.01$) under stimulation throughout the 6 months of the study as compared to baseline values [26]. The pattern of the increase in flow rates revealed an initial profound response followed by a moderate reduction. However, the study patients reported a total reversal of the salivary discomfort they had experienced prior to taking the drug and the high level of satisfaction remained unaltered throughout the 6 months of the study. It was also demonstrated that continuous administration of pilocarpine hydrochloride is essential for the GVHD patients, since an interruption of the drug administration at 2 months resulted in a rapid return to baseline salivary hypofunctional status. Another study [25] demonstrated that pilocarpine hydrochloride normalized the GVHD-induced salivary compositional alterations. The reversal of the induced salivary quantitative and qualitative alterations returned to the saliva its normal biochemical, immunologic and antimicrobial protective properties, which are crucial to GVHD patients in view of their heavy immunosuppression and susceptibility to episodes of overwhelming infection

Underlying mechanism of salivary involvement in GVHD

Prolific research in recent years has significantly enhanced our understanding of the pathogenesis of salivary involvement in GVHD. This contributed mainly in three respects:

- A better understanding of the injurious mechanisms and the role of inflammatory cytokines by which various tissues and organs are affected in GVHD, especially the salivary glands.
- Presented an easy and non-invasive method of monitoring both the disease and its therapy by measuring the induced hyposalivation. This hyposalivation was found to be highly sensitive and correlated well with the severity of the clinical situation.
- Development of new therapeutic regimens to combat the debilitating salivary outcomes of GVHD. This is important in both eliminating the induced suffering and preventing various related health compromises.

Two studies published by Hiroki and co-workers in 1994 and 1996 [21,36] added significantly to our understanding of the cellular and molecular basis of salivary involvement in GVHD. They showed that this involvement is characterized by lymphocyte (mostly T lymphocytes) infiltration into the glandular parenchyma and especially around the secretor ducts. Most of these infiltrating lymphocytes were T cells with a predominance of CD8+ over CD4+. Ductal epithelial cell-associated lymphocytic infiltration expressed HLA-DR, while the expression of adhesion molecules and especially of VCAM-1 was found mostly in endothelial cells but also in epithelial cells. Such periductal infiltration accompanied by salivary parenchymal atrophy and destruction were noted, while the levels of these pathohistologic alterations were found to correlate significantly with the level of induced salivary hypofunction.

MIP-1 = macrophage inflammatory protein 1 alpha
HSP = heat shock protein
TNFα = tumor necrosis factor-alpha
VCAM-1 = vascular cell adhesion molecule 1

Accordingly, a suggested pathogenetic mechanism for salivary gland involvement in GVHD is composed of the following: HLA up-regulation, mononuclear infiltration, and cytokine disregulation [16]. Indeed, recently, various cytokines, including IL-2, IL-6, IFNγ, TNFα and insulin-like growth factor, were shown to influence salivary cell function and survival and the production of salivary immunoglobulin and/or saliva [37]. Therefore, it is possible that a massive lymphocytic infiltration and aberrant production of cytokines are responsible for the described GVHD-induced salivary effects. This results in gland destruction and hypofunction and causes severe xerostomia, which also contributes to the oral manifestations observed in GVHD patients as various salivary protective characteristics are lost [5]. Furthermore, the deleterious effect of hyposalivation is exacerbated by altered salivary composition. The macromolecule content of minor salivary glands, which is significantly different from that of major salivary glands, may explain the presumably contradictory finding of increased salivary immunoglobulin levels in the whole saliva of GVHD patients that was reported recently [24]. This increase in the concentrations of other salivary protecting "players" – total IgA, IgG and EGF – may actually be reduced in total quantity in the oral cavity due to the overwhelming reduction in salivary volume. Moreover, due to the increased viscosity of the saliva, they may not be available at the required mucosal sites where damage occurs but remain ineffective within the saliva itself. Accordingly, the epithelial barriers, which are already severely damaged in GVHD, are left with significantly compromised protection. Nonetheless, it remains controversial whether the relatively low dose of radiotherapy administered prior to bone marrow transplantation is responsible for the observed salivary gland, as was shown in an animal model [15]. In contrast, Brattstrom et al. [38] reported that the salivary glands were profoundly affected in humans exposed to total body irradiation of at least 7.5 Gy prior to bone marrow transplantation. This was demonstrated by an increase in serum amylase levels from a mean of 3.2 mukat/L prior to the irradiation to a mean of 100.3 mukat/L on the day following the irradiation, while 90% of this increase originated from salivary isoamylase.

In summary, the suggested multisite mechanism gains support from the following:

- The differentiated concentration variations of several salivary components as opposed to a similar general wall-to-wall change for all compounds (as would be expected if it were a mere induced reduction of the watery volume, for example).
- Among the altered components, a few, EGF for example, mainly originate in the salivary glands while others, such as total IgG, total IgA and albumin, are serum-borne components. The latter's higher concentrations found in GVHD patients' saliva indicate increased leakage through injured oral mucosa.
- The different (and sometimes opposite) results of various salivary parameters, such as Na and K concentrations, were demonstrated in studies evaluating "pure" secretions of the parotid or the submandibular/sublingual glands vs. those analyzing whole saliva. An increase in salivary sodium may reflect leakage through the oral mucosa and/or injury of the reabsorbing ductal system, while a decrease is probably merely a result of flow rate reduction.

As for the presumable similarity of salivary gland involvement in GVHD and Sjögren's syndrome, it is important to note the finding of Hiroki et al. [37], where two parameters examined were found to be different in the aforementioned diseases:

- In Sjögren's syndrome CD4+ T lymphocytes were predominant while in GVHD there was a slight predominance of CD8+ cells over CD4+ cells. While 10–30% of the infiltrating lymphocytes to the salivary glands in Sjögren's syndrome were B lymphocytes, less than 10% of the infiltrating lymphocytes in GVHD were B lymphocytes.
- The expression of adhesion molecules on the salivary ductal epithelial cells in Sjögren's syndrome, especially VCAM-1, was found to be more profound than in GVHD.

Thus, though it is tempting to posit that both entities are very similar, in light of the immunologic background and the mutual clinical symptoms of xerostomia and xerophtalmia, one should doubt this assumption. Finally, it is important to note that the major salivary immunoglobulin related to protecting both soft and hard tissue, secretory IgA, was reported by Izutsu et al. [16] to be significantly reduced in GVHD patients, and therefore the epithelial barriers that are already severely damaged in GVHD are left with significantly compromised protection.

References

1. Schmitz N, Beksac M, Hasenclever D, et al. Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. *Blood* 2002;100:761–7.
2. Storek J, Gooley T, Siadak M, et al. Allogeneic peripheral blood stem cell transplantation may be associated with a high risk of chronic graft-versus-host-disease. *Blood* 1997;12:4705–9.
3. Rocha V, Wagner JE Jr, Sobocinski KA, et al. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and international bone marrow transplant registry working committee on alternative donor and stem cell sources. *N Engl J Med* 2000;342:1846–54.
4. Aversa F, Tabilio A, Velardi A, et al. Treatment of high-risk acute leukemia with T cell depleted stem cells from related donors with one fully mismatched HLA haplotype. *N Engl J Med* 1998;339:1186–93.
5. Sullivan KM, Shulman HM, Storb R. Chronic graft versus host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. *Blood* 1981;57:267–76.
6. Shimoni A, Nagler A. Non-myeloablative stem cell transplantation (NST): chimerism testing as guidance for immune-therapeutic manipulation. *Leukemia* 2001;15:1967–75.
7. Shulman HM, Sale GE, Lerner KG, et al. Chronic cutaneous graft-versus-host disease in man. *Am J Pathol* 1978;91:545–70.
8. Janin-Mercier A, Devergie A, Van Cauwenberge D, et al. Immunohistologic and ultrastructural study of the sclerotic skin in chronic graft versus host disease in man. *Am J Pathol* 1984;115:296–306.
9. Nagler A, Pines M, Abadi U, et al. Oral tolerization ameliorates liver disorders associated with chronic graft versus host disease in mice. *Hepatology* 2000;13:641–8.
10. Ilan Y, Gotsman I, Pines M, et al. Induction of oral tolerance in splenocyte recipients towards pre-transplant splenocyte antigens ameliorates chronic graft versus host disease in murine model. *Blood* 2000;95:3613–19.
11. Gaziev D, Galimberti M, Lucarelli G, Polchi P. Chronic graft versus host disease: is there an alternative to the conventional treatment? [Review]. *Bone Marrow Transplant* 2000;25:689–96.

12. Hirozane A, Fujikura Y, Sawada T. Analysis of major histocompatibility complex [MHC] class I antigen in the rat salivary gland. *Arch Oral Biol* 1992;37:147–51.
13. Saitoh T, Fujiwara M, Asakura H. Ductal lesions of exocrine glands and insulitis induced by L3T4+ T cells following graft-versus-host reaction due to major histocompatibility complex class II disparity. *Clin Immunol Immunopathol* 1990;57:339-50.
14. Nagler R, Laufer D, Nagler A. Parotid gland dysfunction in a murine model of acute graft versus host disease. *Head Neck* 1998;20:58–62.
15. Nagler RM, Laufer D, Nagler A. Parotid gland dysfunction in an animal model of chronic graft-versus-host disease. *Arch Otolaryngol Head Neck Surg* 1996;122:1057–60.
16. Izutsu KT, Sullivan KM, Schubert MM. Disordered salivary immunoglobulin secretion and sodium transport in human graft-versus-host disease. *Transplant* 1983;35:441–6.
17. Chaushu S, Chaushu G, Garfunkle AA. Immunoglobulins in recipients of bone marrow grafts. Transient secretion of donor-derived salivary IgA following major transplantation of T cell-depleted bone marrow. *Bone Marrow Transplant* 1994;14:925–8.
18. Fox PC, van der Van P, Sonies BC, Weijjenbach JM, Baum BJ. Xerostomia: evaluation of a symptom with increasing significance. *J Am Dent Assoc* 1985;110:519–25.
19. Nagler RM, Nagler A. Sialometrical and sialochemical analysis of patients with chronic graft versus host disease – a prolonged study. *Cancer Invest* 2003;21(1):34–40.
20. Nakhleh RE, Miller W, Snover DC. Significance of mucosal vs. salivary gland changes in lip biopsies in the diagnosis of chronic graft-host-host disease. *Arch Pathol Lab Med* 1989;113:932–4.
21. Hiroki A, Nakamura S, Shinohara M, Oka M. Significance of oral examination in chronic graft-versus-host disease. *J Oral Pathol Med* 1994;23:209–15.
22. Nagler R, Marmary Y, Krausz Y, Chrisin R, Markitziu A, Nagler A. Major salivary gland dysfunction in human acute and chronic graft-versus-host disease (GVHD). *Bone Marrow Transplant* 1996;17:219–24..
23. Norhagen G, Enstrom PE, Bjorkstrand B, Hammarstrom L, Smith CI, Ringden O. Salivary and serum immunoglobulins in recipients of transplanted allogeneic and autologous bone marrow. *Bone Marrow Transplant* 1994;14:229–34.
24. Nagler RM, Nagler A. The effect of pilocarpine on the constituents of saliva in patients with graft vs. host disease. *Arch Oral Biol* 2001;46:689–95.
25. Marti U, Burwen SJ, Jones AL. Biological effects of epidermal growth factor, with emphasis on the gastrointestinal tract and liver: an update. *Hepatology* 1989;9:126–38.
26. Nagler RM, Nagler A. Pilocarpine hydrochloride relieves xerostomia in chronic graft versus host disease patients: a sialometrical study. *Bone Marrow Transplant* 1999;23:1–5.
27. Matsuda Y, Hara J, Osugi Y, Tokimasa S, et al. Serum levels of soluble adhesion molecules in stem cell transplantation-related complications. *Bone Marrow Transplant* 2001;27:977–82.
28. Itoh S, Matsuzaki Y, Kimura T, et al. Suppression of hepatic lesions in a murine graft-versus-host reaction by antibodies against adhesion molecules. *J Hepatol* 2000;32:587–95.
29. Maruya E, Saji H, Seki S, et al. Evidence that CD31, CD49b and CD62L are immunodominant minor histocompatibility agents in HLA identical sibling bone marrow transplants. *Blood* 1998;92:2169–76.
30. Cavet J, Dickinson AM, Norden J, Taylor PR, Jackson GH, Middleton L. Interferon-gamma and interleukin-6 gene polymorphous associated with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. *Blood* 2001;98:1594–600.
31. Panoskaltsis-Mortari A, Taylor PA, Rubin JS, et al. Keratinocyte growth factor facilitates alloengraftment and ameliorates graft-versus-host disease in mice by a mechanism independent of repair of conditioning-induced tissue injury. *Blood* 2000;96:4350–6.
32. Higaki Y, Yamado O, Okamura T, Mizoguchi H, Kawashima M. Granzyme-B-containing lymphocyte involvement in epidermal injury in graft-versus-host disease. *Dermatology* 2001;202:94–8.
33. Serody JS, Burkett SE, Panoskaltsis-Mortari A, et al. T-lymphocyte production of macrophage inflammatory protein 1alpha is critical to the recruitment of CD8(+) T cells to the liver, lung and spleen during graft-versus-host disease. *Blood* 2000;96(9):2973–80.
34. Goral J, Mathews HL, Nadler SG, Clancy J. Reduced levels of Hsp 70 result in a therapeutic effect of 15-deoxyspergualin on acute graft-versus-host disease in (DA x LE) F1 rats. *Immunobiology* 2000;303:254–66.
35. Kichian K, Nestel FP, Kim, D, Ponka P, Lapp WS. IL-20 p40 messenger RNA expression in target organs during acute graft-versus-host disease. Possible involvement of IFN-γ. *J Immunol* 1996;157:2851–6.
36. Hiroki A, Nakamura S, Shinohara M, et al. A comparison of glandular involvement between chronic graft-versus-host disease and Sjögren's syndrome. *Int J Maxillofac Surg* 1996;25:298–307.
37. Nagler A, Nagler RM, Ackerstein S, Levy S, Marmary Y. Major salivary gland dysfunction in patients with hematological malignancies receiving interleukin-2 based immunotherapy post autologous blood stem cell transplantation [Abstract]. *Bone Marrow Transplant* 1997;20:575–80.
38. Brattstrom C, Tollemar J, Ringden O, Bergstrom K, Tyden G. Isoamylase levels in bone marrow transplant patients are affected by total body irradiation and not by graft-versus-host disease. *Transplant Int* 1991;4(2):96–8.

Correspondence: Dr. R.M. Nagler, Oral and Maxillofacial Surgery Department, Rambam Medical Center, Haifa 31096, Israel.
Phone: (972-4) 644-2003
Fax: (972-4) 654-295
email: nagler@tx.technion.ac.il