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Opportunistic autoimmune disorders:

From immunotherapy to immune dysregulation

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Abstract

Rapid advances in our understanding of the immune network have led to treatment modalities for malignancies and autoimmune diseases based on modulation of the immune response. Yet therapeutic modulation has resulted in immune dysregulation and opportunistic autoimmune sequelae, despite prescreening efforts in clinical trials. This review focuses on recent clinical data on opportunistic autoimmune disorders arising from three immunotherapeutic modalities: (1) systemic immunomodulators, including interferon-α (also used to treat hepatitis C patients) and interferon-β; (2) monoclonal antibodies to CTLA-4 and CD52, and (3) hematopoietic stem cell transplantation. Uncategorized predisposing factors in these patients include major histocompatibility complex and gender genetics, prevalence of different autoimmune diseases, prior chemotherapy, underlying disorder (e.g., hepatitis C), and preconditioning regimens as part of organ and stem cell transplants. Not unexpectedly, the prevalent autoimmune thyroid disease surfaced frequently. Our combination models to study the balance between thyroid autoimmunity and tumor immunity upon regulatory T-cell perturbation are briefly described.

Keywords

opportunistic autoimmunity; immunotherapeutic sequelae; immunotherapy; autoimmunity; immune dysregulation

Introduction

A prominent feature in recent clinical trials has been to target the immune system, either with systemic immunomodulators or specific monoclonal antibodies (mAbs), in order to improve the efficacy of immunotherapy for cancer and autoimmune diseases. Despite prescreening to exclude patients with pre-existing autoimmune diseases, cancer therapy aimed at suppressing regulatory T (Treg) cell function or activating effector T-cell function by immunomodulation not only enhances antitumor immunity but also triggers autoimmunity. Even targeting autoimmune diseases with cytokines or anti-T- or anti-B-cell

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agents can evoke secondary autoimmune diseases, most likely in individuals who may already be genetically autoimmunity-prone.

It has long been recognized that the use of immunosuppressive agents to treat cancer, transplant rejection, and autoimmune diseases predisposes individuals to opportunistic infections and certain malignancies. Likewise, immunoenhancing agents can stimulate untoward responses that negate the therapeutic goal and result in the development of opportunistic autoimmune diseases. This review will focus only on opportunistic autoimmune disorders, at times extremely severe, that arise from perturbation of the immune network during immunotherapy and that may coexist with infections and other side effects.

Systemic immunomodulators

Systemic immunomodulation, also known as adjuvant therapy, has been a treatment modality in a variety of clinical diseases to boost the immune response even though the antigens are not always known or are ill defined. Often the response rate to systemic immunomodulation is unpredictable and efficacy is below 50%. Moreover, prolonged treatment provides continual stimuli to autoreactive T cells, triggering autoimmune sequelae.

Interferon-α

Interferon-α (IFN-α) is a type I interferon that has been widely used as a therapeutic agent.[1] The binding of IFN-α to its receptors activate various signaling pathways, including the Janus kinase and signal transducer and activator of transcription (JAK-STAT) pathway, the Crk-pathway, the insulin receptor substrate (IRS) signaling pathway, and the mitogen-activated protein (MAP) kinase pathway.[2,3] More than two dozen interferon-induced proteins have been identified.[4] In the past several decades, IFN-α has emerged as a major therapeutic modality for several malignant and nonmalignant diseases, including hepatitis C, carcinoid tumors, hairy cell leukemia, and Kaposi's sarcoma.[4–8] However, the therapeutic use of IFN-α is limited by its wide side-effect profile, ranging from influenza-like symptoms to hematologic effects. These side effects cumulatively can lead to dose reductions in up to 40% of patients and drug discontinuation in up to 14% of patients.[9]

IFN-α therapy and autoimmunity

Not unexpectedly, one of the most common side effects of IFN-α therapy is development of several autoimmune conditions, such as autoimmune thyroid disease (ATD), systemic lupus erythematosus (SLE), and type 1 diabetes (T1D).[10] Interferon IFN-α-induced autoimmunity can manifest by the development of autoantibodies alone or by the full clinical picture of the disease. Interestingly, autoimmune diseases triggered by IFN-α include both T-cell-mediated (e.g., Hashimoto's thyroiditis [HT] and T1D[11]) and antibody-mediated (e.g., SLE[12] and Graves' disease [GD][13]) diseases. Thus, IFN-α may exert a general stimulatory effect on the immune system in individuals genetically predisposed to a specific autoimmune condition. For example, IFN-α may activate an inflammatory response by a bystander mechanism, which may then develop into cellular or humoral autoimmune response depending on the genetic susceptibility of the individual. Indeed, several studies have suggested a genetic predisposition to IFN-α-induced autoimmunity (reviewed in Ref. 14).

SLE is a prototypic systemic autoimmune disease, and several studies have shown an association between IFN-α therapy and SLE.[15] Okanoue and colleagues[10] followed 677 patients with chronic hepatitic C who completed a full course of IFN-α for 22 weeks. Of the 677 patients, 92 (13.6%) had positive anti-nuclear antibodies (ANA) and 105 (15.5%) has positive anti-DNA antibodies prior to IFN-α therapy. Of the patients with positive

antibodies, ANA titers increased in 39.1%, and anti-DNA titers increased in 27.6% during IFN-α therapy. One patient developed an SLE-like syndrome, which resolved 6 months later. In addition, 12 patients (1.8%) developed hyperthyroidism, and six patients (0.9%) developed hypothyroidism. Overall, the frequency of clinical SLE in patients on IFN-α therapy is about 1% (reviewed in Ref. 15). Moreover, Mathian and colleagues showed that lupus-prone NZBxNZW F_1 mice injected with murine IFN-α in an adenovirus vector developed severe SLE with 100% mortality.[16] Severe cases of SLE were also reported in humans following IFN-α therapy for chronic hepatitis C virus infection.[12] Other autoimmune disorders reported in patients receiving IFN-α therapy include rheumatoid arthritis (RA), Sjogren's syndrome, polymyositis, vitiligo, pernicious anemia, and T1D.[15,17,18] Moreover, one group reported the development of a plethora of antibodies to various epithelial cells in carcinoid patients receiving IFN-α, including antibodies to bile epithelium, pancreatic secretory ducts, fallopian tube, and distal renal tubuli.[19]

IFN-α-induced thyroiditis in hepatitis C patients

By far the strongest association of IFN-α therapy with autoimmunity is with ATD. The association between IFN-α and thyroid disease was recognized as early as 1985 in patients being treated with IFN-α for carcinoid tumors and breast cancer.[6,8] Since then numerous studies have reported a high incidence of thyroid disease in patients treated with IFN-α.[20,21] Intriguingly, IFN-α-induced thyroiditis (IIT) can manifest both as autoimmune thyroiditis and non-autoimmune thyroiditis.[13] The most common clinical manifestation of autoimmune IIT is HT (reviewed in Ref. 13). Most studies have shown that the presence of thyroid antibodies (TAbs) prior to the initiation of IFN-α therapy is a significant risk factor for the development of IIT manifesting as HT.[22] Therefore, screening for TAbs prior to the initiation of IFN-α therapy can help assess the risk of developing HT.[13]

Less commonly, treatment with IFN-α can result in the development of GD (reviewed in Ref. 13). A retrospective review of 321 patients with hepatitis B or C treated with IFN-α found 10 patients who developed thyrotoxicosis, and six of them were diagnosed with GD based on diffusely increased uptake on thyroid scintigraphy as well as positive thyroid-stimulating antibodies.[23] In most reported cases of GD developing secondary to IFN-α therapy, the disease did not go into remission when IFN-α therapy was completed or stopped,[23–25] suggesting that IFN-α triggered the disease in genetically predisposed individuals. Most commonly, patients receiving IFN-α therapy develop TAbs without clinical disease.[13] TAbs can develop *de novo* during IFN-α, or IFN-α can cause a significant increase in TAb levels in individuals who were positive for TAb prior to IFN-α therapy. The incidence of *de novo* development of TAbs by IFN-α is up to 40%.[13] Not unexpectedly, in view of the higher frequency of ATD in women, the development of TAbs in IFN-α-treated patients was significantly higher in women compared to men (14.8% versus 1%; $P < 0.01$). The majority of individuals who develop *de novo* TAbs on IFN-α therapy remain TAb-positive after the end of treatment.[26]

In up to 50% of IIT patients, the thyroiditis is not autoimmune, i.e., it is not associated with the production of TAbs or other signs of thyroid autoimmunity.[22,23,27] Non-autoimmune IIT usually manifests as destructive thyroiditis, a self-limited inflammatory disease characterized by sudden onset of hyperthyroidism, followed by a hypothyroid phase, and eventually resolves with normalized thyroid functions within several weeks to months.[28] More than 50% of IIT patients with thyrotoxicosis have destructive thyroiditis, while the remainder have GD.[21–23,25,27,29] Another form of non-autoimmune IIT is hypothyroidism without TAbs.[10,25,30] In many of these cases, the hypothyroidism is transient.[13]

IIT in other conditions

IFN-α therapy has also been noted to cause autoimmunity when used as therapy for malignancies, such as breast cancer, carcinoid tumors, and hematologic malignancies.[5,6,31–33] Fentiman and colleagues[31] reported in 1988 that 50% of women treated with IFN-α as adjuvant therapy for breast cancer developed positive TAbs and 30% developed ATD. All patients who developed ATD had positive TAbs before or during treatment.[31] Similarly, Ronnblom and colleagues found that 13% of patients treated with IFN-α for carcinoid tumors developed thyroid disease.[5] As in patients with hepatitis C, the risk of developing ATD was significantly higher in patients with pre-existing positive TAbs. Even though originally it was thought that impurities in the leukocyte-derived IFN-α preparations caused the induction of autoimmunity, high frequency of thyroid autoimmunity was seen both with leukocyte-derived and recombinant IFN-α, thus excluding this possibility.[5]

Interestingly, recent data suggest that the appearance of autoantibodies and clinical autoimmunity in melanoma patients treated with IFN-α might be a good prognostic sign for improved antitumor activity.[34,35] Of 134 patients, 20 (14.9%) patients were diagnosed with autoimmune thyroiditis and one also developed RA; the dual autoimmune sequelae resulted in discontinuation of therapy.[34] Of 200 other melanoma patients, 52 (26%) developed TAbs, ANA, and other manifestations of autoimmunity, and 6% of patients overall developed hypothyroidism and hyperthyroidism.[35] TAbs were also the most frequently observed, suggesting that had the sex distribution of female: male been as high as ATD in the general population (6:1 instead of ~1:1 in the treated patients), the incidence and severity might have been greater.

Interferon-β (IFN-β) has been used to treat patients with relapsing-remitting multiple sclerosis (MS).[36] Intriguingly, even though IFN-β improves the clinical outcome of MS patients, it induces thyroid autoimmunity in up to 19% of treated MS patients.[27,36–38] Apparently, INF-β is immunosuppressive for MS-specific T-cell clones but is stimulatory for thyroid-specific clones. It is also possible that the thyroid dysfunction seen in IFN-β on treated patients is a result of a direct toxic effect of interferon on the thyroid (see below).

Pathogenesis of IIT

Epidemiologic data support a genetic predisposition for IIT. Similar to ATD, IIT is more common in females than in males,[20] and there are variations in the prevalence of IIT among ethnic groups, suggesting genetic predisposition.[39] Additional evidence for genetic predisposition to IIT comes from studies that have suggested that IFN-α accelerates thyroiditis in a thyroiditis-prone mouse model, the NOD-H2^{h4} mouse.[40] Two studies showed associations of IIT with certain *HLA* alleles.[41,42]

The mechanisms by which IFN-α triggers IIT in genetically predisposed individuals are still unknown. However, recent findings suggested that both immune-mediated and direct thyroid-toxic effects of IFN-α play a role in the etiology of IIT. IFN-α exerts various effects on the immune system, many of which might be implicated in the development of autoimmunity. IFN-α receptor activation results in activation of the JAK-STAT pathway,[43] leading to activation of a large number of IFN-α-stimulated genes (ISGs), including cytokine and adhesion molecule genes.[44,45] These combined effects could trigger autoimmunity by bystander mechanisms.[46] One of the cardinal effects of IFN-α is to increase MHC class I antigen expression, which is associated with activation of cytotoxic T cells, leading to tissue damage and inflammation.[44] IFN-α could induce cell-mediated autoimmunity by shifting the immune response to a T helper (Th)1-mediated pattern.[47] Indeed, it was recently reported that hepatitis C patients that developed IIT showed Th1

polarization of their innate immune response.[48] However, in some patients with IIT, the clinical picture is that of GD, which is generally believed to be a Th2-mediated disease.[49,50] Actually, the initiation of GD is likely to be Th1-mediated.[51] Additional effects of IFN-α that could contribute to the development of autoimmunity include enhancement of the activity of lymphocytes, macrophages, and natural killer (NK) cells[1,44,52,53] as well as neutrophil and monocyte activation.[44] IFN-α can induce the release of cytokines, such as IL-6,[44] a cytokine that has been associated with autoimmune thyroiditis.[54]

As up to 50% of patients with IIT have non-autoimmune thyroiditis, it is likely that IFN-α exerts direct effects on the thyroid. Preliminary data suggested that incubation of thyroid cells with IFN-α induced thyroid cell death by nonapoptotic mechanisms.[55]

Interleukin-2

IL-2 is used for the treatment of metastatic melanoma. Similar to IFN-α, IL-2 has been reported to induce the development of several autoimmune conditions, most notably ATD (reviewed in Ref. 56). In addition, patients receiving IL-2 were reported to develop diabetes[57] and RA.[58] While the mechanisms are unknown, they may be similar to those leading to INF-α-induced autoimmunity where the inflammatory response is induced by bystander activation of tissue-resident T cells.

Flt3 ligand

To enhance the immune response to a peptide vaccine derived from a family member of human epidermal growth factor receptor (Her-2/rat neu) in prostate cancer patients, human recombinant flt3 ligand, a growth/differentiation stimulator for dendritic cells, was used as a systemic adjuvant.[59] Among 15 patients, two developed elevated levels of thyroid-stimulating hormone (TSH) with hypothyroid-like symptoms. Retrospectively, pretreatment sera from the two subjects were screened for reactivity to thyroid antigens, and TAbs to thyroglobulin (Tg) and/or thyroid peroxidase were detected or elevated. A third also exhibited increased TSH level without symptoms. Thus, there might have been underlying subclinical hypothyroidism aggravated by adjuvant therapy. It is unknown if any others in the trial also had an autoantibody response that might have been initiated or elevated during treatment with subsequent development of ATD.

Summary

Systemic immunomodulatory drugs are being used as therapeutic modalities with increasing frequency. These new agents show promise and have powerful therapeutic effects in malignant disorders, infectious diseases, and autoimmune diseases. However, not unexpectedly, systemic immunomodulation frequently results in untoward effects, most notably autoimmune phenomena. Thus, IFN-α therapy has been reported to trigger thyroid autoimmunity, TID, and SLE plus a variety of other autoantibodies.[13] Intriguingly, thyroiditis is the most common autoimmune side effect of the three main systemic immunomodulators currently in clinical use (IFN-α, IL-2, and flt3 ligand). Moreover, the thyroiditis may be non-autoimmune (e.g., destructive thyroiditis), suggesting a direct thyroid-toxic effect. The most studied systemic immunomodulator is IFN-α, which is associated with clinical thyroiditis in up to 15% of hepatitis C patients and with thyroid antibody positivity in up to 40%.[13] The mechanisms leading to the development of IIT are beginning to be unraveled. The hepatitis C virus itself plays a role as well as genetic predisposition. Recent data suggest that IFN-α precipitates thyroiditis by both immunomodulatory mechanisms and direct thyroid-toxic effects.[14]

Monoclonal antibodies targeting the T- and/or B-cell compartment

The 21st century ushered in a large number of U.S. Food and Drug Administration-approved mAbs as therapeutics for both cancer and autoimmune diseases.[60,61] A few more of these humanized recombinant mAbs are undergoing clinical trials, and some are being used for renal transplantation. Although cytokine therapy has been approved for use in cancer, MS, and hepatitis C infection (Systemic immunomodulator), the response rate in most instances is only approximately 30–50% and is often accompanied by severe side effects stemming from systemic stimulation to the immune system. Thus, the goal of immunotherapy continues to entail specific targeting of the immune network, judged to be involved in suppressing or enhancing the necessary response. This section will deal with recent mAb therapy trials that have included sufficient numbers of patients to evaluate the increased risk of autoimmune disorders.

Cytotoxic T lymphocyte-associated antigen 4 mAb

Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), a T-cell marker, is upregulated during T-cell-mediated immune response and functions as a negative regulator by binding to the B7 family costimulatory molecules on antigen-presenting cells with higher affinity than CD28. It is also constitutively expressed on Tregs and is critical to the proper functioning of $Foxp3^+$ Tregs;[62] indeed, CTLA-$4^{-/-}$ mice develop severe multi-organ autoimmunity.[63,64] Anti-CTLA-4 was shown recently to interfere with the activation of $CD4^+CD25^+Foxp3^+$ Tregs by Tg to mediate resistance to experimental autoimmune thyroiditis (EAT).[65] Thus, using CTLA-4 mAb to treat cancer could serve to prevent tumor antigens from activating Tregs, which could suppress antitumor response, as well as to dampen negative signaling of an ongoing T-cell response.[66]

Clinical trials reveal unusual spectrum of autoimmune sequelae

A recent review summarized the responses of 139 metastatic melanoma patients who were treated with repeated doses of a CTLA-4 mAb (ipilimumab) while receiving a peptide vaccine, with an average follow-up time of 2–4 years.[67] The frequency of adverse immune events classified as grade I/II (45%) included dermatitis with pruritus and those classified as grade III/IV (36%) included the more problematic enterocolitis (17%) and hypophysitis (9%), resulting in intervention or cessation of therapy. Dissecting the relationship between adverse immune events and antitumor response, grade III/IV adverse events were observed in 28% (14 of 50) of patients with an objective response. Of 36 patients experiencing grade I/II adverse events, eight (22%) displayed a partial response. The total response rate of 17% (23 of 139) included one complete responder without adverse events and two complete and 20 partial responders with adverse autoimmune disorders. Thus, adverse autoimmune responses were judged to be associated with improved antitumor response, as also reported earlier.[68] However, of the 86 patients with mild to severe autoimmune symptoms, 74% (64 of 86) showed no objective improvement. Clearly, autoimmune disorders are not good predictors of improvement in anti-CTLA-4 immunotherapy.

Another more recently developed humanized CTLA-4 mAb (tremelimumab, CP-675,206) with a longer half-life of elimination has undergone phase I and phase II trials in patients with melanoma or renal cell or colon carcinoma.[69–71] Adverse immune events were dose-related. The autoimmune manifestations also included panhypopituitarism, hypothyroidism or hyperthyroidism, enterocolitis, vitiligo, and other autoimmune phenomena. Another larger phase III registration trial had 324 patients but was halted about 1 year later because it did not reach the overall survival objective to warrant the multiple adverse toxicities.[71,72] Besides the usual side effects of diarrhea, pruritus, and vitiligo (in conjunction with tumor

vaccine), there were pituitary or adrenal gland dysfunction (3%), thyroid disease (4%), and treatment-related deaths.[71]

ATD, including GD and HT, is among the most common autoimmune conditions, suggesting a relatively common genetic predisposition to ATD.[73] Therefore, increased risk of ATD after therapy with systemic modulators, which could trigger autoimmunity in susceptible individuals, has often been observed (Systemic immunomodulators). It should not be surprising that CTLA-4 mAb therapy would evoke ATD as it blocks Treg activation, which maintains a state of self-tolerance. In the EAT model, anti-CTLA-4 co-administration with tolerogenic mouse Tg blocks induction of tolerance.[65] The incidence and severity of autoimmune thyroid disorders might have been higher had the emphasis in these trials not been *HLA-A*0201* individuals because of the human leukocyte antigen (HLA)-A2-restricted peptide vaccine. There was insufficient attention to patients' to *HLA* class II genes with known association with thyroid and other diseases. Furthermore, the melanoma patients had a 1:2, female: male ratio, whereas ATD is 5–10 times more prevalent in females. The patients had all been prescreened for the presence of autoimmune disorders, and any individual with such disorders, manifested during prior chemotherapy or immunotherapy, would have been excluded.[67] The propensity for predominant enterocolitis in the CTLA-4 mAb trials could have been magnified from prior treatment but was difficult to exclude.[68]

On the other hand, the incidence of autoimmune hypophysitis of 9% (13 of 139) after ipilimumab[67] and 3% after tremelimumab[71] treatment was higher than expected. The autoimmune nature with multi-organ endocrine dysfunction was documented in an earlier report that included patients with metastatic melanoma and renal cell cancer; all eight patients with autoimmune hypophysitis were male.[74] Although autoimmune hypophysitis has been observed after treatment with IFN-α,[75] it is a rare autoimmune disease.[76]

Summary

The cancer patients enrolled in these trials had all undergone prior treatments with a variety of agents, including chemotherapy, IL-2, or IFN-α, which might have predisposed them to further autoimmune disorders, despite prescreening to exclude those with ongoing autoimmune diseases. The patient population was selected on the basis of possessing the *HLA-A*0201* allele. Their *HLA* class II genes were undetermined as were the *CTLA-4* gene polymorphisms known to predispose to autoimmunity.[77] Moreover, the melanoma patients had a reverse gender ratio (female: male of ~1:2), compared to the ratio observed in ATD and RA (female: male of ~6–8:1).[73] Even so, ATD was a prominent sequela in these male-dominated melanoma clinical trials.

The occurrence of both ATD and hypophysitis suggests *de novo* generation of sequelae associated with CTLA-4 blockage of Treg-mediated suppression. If such blockade also stimulated $CD4^+$ and $CD8^+$ effector cell function while blocking negative signaling to T cells, as reported in melanoma patients given CTLA-4 mAb where HLADR expression was also upregulated,[78] the stimulation could also extend to other autoreactive T cells.

Combination models to study the balance between autoimmunity and tumor immunity upon Treg perturbation

We recently examined four murine models combining EAT and breast cancer vaccine protocols under the umbrella of Treg depletion and MHC class II gene influence. In each combination model, Treg influence is either well established, as with EAT in both susceptible and resistant strains, or can be studied to enhance vaccine efficacy in wild-type mice or mice transgenic for Her2/neu breast cancer antigen. As reviewed recently,[79] the first model was an established breast cancer model in an EAT-resistant ($H2^d$) strain in which

EAT and antitumor immunity were induced concurrently at the time of Treg depletion with a CD25 mAb.[80] Treg depletion enhanced tumor regression and facilitated thyroiditis induction with mouse Tg, with or without LPS as adjuvant. Interestingly, immune responses to neu or mouse Tg were greater than uncombined control mice given mouse Tg or tumor separately, suggesting that ongoing tumor regression and autoimmune response provided additional mutual stimuli.

In the second model, we investigated if antitumor response was independent of EAT susceptibility. An HLA-DR3 transgene was introduced into EAT-resistant B6 (*H2A*^{b}) mice or Her-2 transgenic B6 mice to provide a susceptibility allele for mouse Tg.[81] The resultant Her-2xDR3 F_1 mice expressed both mouse A^b and human DR3 and were tolerant to both mTg and Her-2.[82] To induce tumor rejection in Her-2xDR3 mice, Treg depletion must be followed by electrovaccination with Her-2 and mouse pGM-CSF DNA. Tumor rejection was similar in Her-2 transgenic mice expressing either A^b or A^b/DR3, but EAT induction was enhanced by the presence of DR3, indicating that regulation of Her-2 immunity is independent of DR3 expression.

In the third model, we observed that tumor immunity in rat neu transgenic (NeuT) BALB/c strain likewise required both Treg depletion and subsequent neu DNA vaccination.[83] Moreover, protection from spontaneous tumorigenesis for over a year was observed in 58% of protected mice, whereas untreated BALB NeuT females developed spontaneous tumors at 17–19 weeks. Mild EAT development after injecting mouse Tg without LPS was as expected in the EAT-resistant BALB NeuT strain, but in tumor-regressing mice there were detectable increases in IFN-γ-producing T-cell responses to both neu and mouse Tg and greater thyroid destruction when LPS adjuvant was used. Thus, mutual stimulation of neu and mTg reactivity during tumor regression was again observed.

In the fourth model, an EAT-susceptible CBA (*H2*^{k}) strain was used to simulate patients with predisposition to autoimmune disease whose risk was further compounded by therapy targeting the T-cell compartment. Studies in this EAT-susceptible strain have shown that naturally existing Tregs maintain self-tolerance and their presence are required for further strengthening of antigen-specific resistance to EAT induction.[65,84] Using a tumor variant derived from a spontaneous mammary adenocarcinoma, tumor protection was induced by Treg depletion followed by irradiated whole-cell vaccine.[79] Repeated mouse Tg doses were given without adjuvant LPS to simulate the physiologic release of circulatory Tg in all individuals. The majority of mice developed moderate thyroid destruction. This model combining tumor immunity and EAT susceptibility has the potential to examine the extended influence of perturbation and modulation of the immune network, tilting the balance toward immunotherapy.

Models combining autoimmunity and antitumor immunity and those with EAT alone show that Treg influence does not supersede MHC class II restriction, as discussed elsewhere,[85] and that breast cancer immunity and EAT are regulated independently. However, after Treg depletion, concurrent induction of EAT and tumor immunity followed by regression can provide mutual stimuli to each regimen. Clearly any tumor immunity-enhancing protocol must seriously consider the extent of developing autoimmune sequelae.

CD52 mAb

CD52 mAb (alemtuzumab, Campath-1H), a humanized mAb, targets CD52 and depletes $CD52^+$ cells. As reviewed recently,[86] CD52 antigen has been found on most leukocytes, including activated T and B cells, antigen-presenting cells, as well as tumor cells derived from such cells. Since its early and continuing use in renal transplantation,[87] alemtuzumab has undergone a variety of clinical trials as an immunotherapeutic and its efficacy depends

on the type of disease and the length/frequency of administration. At times, its success rate is tempered by the appearance of secondary autoimmune diseases, especially as the CD52 marker is so ubiquitous in the immune network, and its role is incompletely understood.

As therapy for multiple sclerosis

IFN-β iscurrentlyinuseasasystemicimmunomodulator for progressive or relapsing-remitting MS.[88] The early concern that IFN-β evoked *de novo* autoimmune thyroid and hepatic dysfunctions was reviewed in prospective studies.[27,37,89,90] In contrast to IFN-α, thyroid dysfunction was less frequent (8.3% including both hyperthyroidism and hypothyroidism and autoantibody positivity), whereas hepatic dysfunction was significantly increased (37.5%).[89] Although neutralizing antibody formation reduces the effect, IFN-β remains a recommended immunotherapeutic agent.[88,89] Despite the mostly treatable, opportunistic, autoimmune disorders, *de novo* or subclinical, the clinical relapse rate apparently is only reduced by INF-β therapy to about one-third, resulting in the continuous quest for other immunotherapeutic agents.[91]

One such immunotherapeutic agent is alemtuzumab, which was evaluated in MS patients over the period 1991–2002 and the follow-up findings from 58 patients were summarized in 2006.[92] The earlier report was on 27 patients with progressive MS treated with five consecutive days of alemtuzumab, which depleted 95% of the circulating lymphocytes.[93] Patients remained lymphopenic with CD4 and CD8 T-cell values at 30–40% of pretreatment levels for at least 18 months. Nine of 27 patients developed GD at 6–31 months post treatment, with two further developing Graves' ophthalmopathy. A few also developed autoantibodies to muscle and nuclear antigens. In hopes of interrupting demyelination and improving later disability seen in treated progressive cases, a multicenter phase II trial comparing alemtuzumab and IFN-β-1a in yet-untreated early MS patients was initiated in 2002–2004 and the findings were recently summarized.[94] Most patients received two of the three annual cycles of alemtuzumab, but 75% were precluded from the third cycle because of three cases of autoimmune thrombocytopenic purpura, resulting in one death. In addition to infection, adverse autoimmune sequelae in the alemtuzumab- (216) versus IFN-β-1a- (107) treated patients were thyroid disorders (23 versus 3%) and immune thrombocytopenic purpura (3 versus 1%).

Thus, ATD continues to be a prominent opportunistic autoimmune disorder in alemtuzumab-treated MS patients, whether alemtuzumab is given within the first week or at one to three annual cycles. Despite prescreening to exclude those with TAbs, with particular attention to TSH receptor antibodies, the incidence of ATD was much higher than IFN-β-1a treatment and rivaled that after IFN-α treatment in hepatitis C patients wherein there are additional predisposing factors (Systemic immunomodulators). Of note, the MS patients were 64% female, which might have increased their predisposition to thyroid autoimmunity. With more patients in the Coles and colleagues report,[94] both hyperthyroid and hypothyroid cases were seen and no difference was discernible between the 12-mg versus 24-mg dose. Gastrointestinal symptoms were also quite high, some of which may have been autoimmune in nature.

Alemtuzumab has also been tested in therapy for various autoimmune diseases, including MS as a conditioning regimen for autologous hematopoietic stem cell transplant (HSCT).[95] Again, opportunistic (secondary) autoimmune sequelae occurred, with discontinuation of therapy in some patients (Hematopoietic stem cell transplant).[96]

As induction therapy in renal transplantation

Employing the principle of perioperative doses of polyclonal rabbit anti-thymocyte immunoglobulin (ATG) at the time of renal transplantation to reduce T-cell number and maintenance immunosuppressants, alemtuzumab (5 patients) was tested with HLA-nonidentical living donor grafts along with depleting ATG (5 patients) and nondepleting anti-CD25[97] (daclizumab; 3 patients).[98] Maintenance immunosuppression was found necessary to prevent acute rejection episodes after profound T-cell depletion. Thus, the remaining recipient T cells were capable of mediating alloimmunity, albeit at a reduced level. The short-term alemtuzumab treatment and maintenance immunosuppression likely inhibit autoreactive T-cell response, limiting autoimmune dysfunction. On the other hand, in another report a patient that did not receive initial maintenance suppression after alemtuzumab induction therapy developed GD 4 years later,[99] suggesting that, similar to MS patients, self-tolerance was difficult to maintain and immunosuppression could inhibit activation of Tregs as well as autoreactive T cells until other predisposing factors regain influence.

As therapy for anti-neutrophil cytoplasm Ab-associated vasculitis

From 1991–1999, 71 patients (female: male ratio ~1:1) with refractory/relapsing anti-neutrophil cytoplasm Ab-associated vasculitis (AASV) were treated with alemtuzumab, some with several repeats, and followed for a median of 5 years.[100] There were 60 patients with initial remission, but 43 patients relapsed and 31 died. Adverse events were highest with infection in 28 patients, malignancy in three patients, and thyroid disease in eight patients. Again, it is unknown if higher female: male ratio and lower death rate would lead to greater incidence of the prevalent autoimmune thyroid disorders.

As therapy for rheumatoid arthritis

There are a few options for treating RA, including those directed toward inflammatory molecules, such as anti-tumor necrosis factor (TNF)-α (infliximab) and IL-1 receptor antagonist, and those against B cells, such as anti-CD20 (rituximab).[61] A 12-year follow-up of 53 patients treated with alemtuzumab revealed that 50% (27 patients) died, which was not different from the death rate of similar RA patients.[101] No other autoimmune disorders were reported with the treatment; however, the lack of improvement over control subjects would not be conducive to its widespread use when other combination therapy is available with less risk.[102]

As therapy for B-cell chronic lymphocytic leukemia

Alemtuzumab has been approved for therapy of B-cell chronic lymphocytic leukemia (B-CLL) for about a decade. Reporting on 23 patients, those without underlying autoimmunity did not develop autoimmune complications.[103] A comprehensive review of other studies showed that opportunistic or reactivated infections were frequent and a major concern, overshadowing other adverse events, such as autoimmune thrombocytopenia and hemolytic anemia, particularly seen if prior therapy had been administered.[86] The general consensus was that sub-cutaneous administration rather than intravenous administration of alemtuzumab reduced the initial infusion-induced complications, which may be related to rapid pro-inflammatory cytokine release.[104]

Alinari and colleagues[86] also reviewed the feasibility studies of alemtuzumab preconditioning in B-CLL patients prior to harvest for autologous HSCT to prevent graft contamination of tumor cells. Furthermore, to reduce graft-versus-host disease (GVHD), alemtuzumab has been tested as part of the preconditioning regimen prior to allogeneic HSCT. The benefit analyses from several studies for allogeneic HSCT are thus far

inconclusive and await further follow-up. Whether autoimmune disorders will develop subsequently is also unknown.

Summary

Since the development of alemtuzumab in the 1990s, it has been used in a variety of clinical conditions stemming from perturbation of the immune network, in addition to renal transplantation. In these patients, opportunistic autoimmune disorders invariably appear more often than in the general population. We have only examined clinical trials that have amassed a relatively large group of patients. Interspersed are a number of reports examining its mechanisms of action as an immunotherapeutic agent. However, because its mode and length of administration are varied and it depletes many different cell types that participate in the immune network and because the patients have different predisposing factors, it is unlikely that uniform conclusions can be drawn soon. There is consensus that the numerous $CD52^+$ cells are reduced severely and recovery time is very slow, as seen with the $CD4^+$ T-cell subset. This population could include Tregs as well as autoreactive T cells; both naive and effector $CD4^+$ cells have been implicated. While T cells mediating underlying subclinical autoimmune disease could survive CD52 mAb treatment and remain as memory-like T-cell phenotype, as described after perioperative dosing of renal transplant patients,[98] these cells could become activated by the cytokines IL-6, TNF-α, and IFN-γ, released from NK cells upon repeated dosing.[104]

IL-6 has been shown to be released by IL-1β administration, which interferes with tolerance induction with mouse Tg in EAT, and TNF-α release could also be implicated after IL-1β injection.[105] It is possible that the protocol of annual cycle of CD52 mAb treatment to MS patients in the phase II trial[94] led to a second release of pro-inflammatory cytokines. The cytokines both stimulated autoreactive T cells and interfered with tolerance maintenance by Tregs, as reported for EAT,[65,105] contributing to the high incidence of ATD. The reduction by alemtuzumab of dendritic cells, which participate in stimulating Tg-specific $CD4^+CD25^+$ Tregs,[106] could also contribute to enhanced ATD. While Tregs might be stimulated or expanded *in vitro* by CD52 mAb,[107] the slow recovery of Tregs as part of the $CD4^+$ T-cell repertoire after alemtuzumab treatment would make such stimulation difficult.

Hematopoietic stem cell transplant

Autologous and allogeneic HSCT have both been used to treat lymphoid malignancies and autoimmune diseases. Some mAbs are used as lymphoid ablative conditioning as well as purging of tumor cells in the host prior to HSCT. For autoimmune diseases, the intent is to clear the host of pathogenic effector T and B cells, thereby replacing the lymphoid repertoire. In many instances, lymphoid ablation has largely relied upon total body irradiation and/or chemotherapeutic agents, such as cyclophosphamide.

HCST in lymphoid malignancies

Appropriate preconditioning in conjunction with autologous HSCT has helped combat lymphoid malignancies; the resultant autoimmune phenomenon of graft-versus-lymphoma (GVL) has proven beneficial. However, the same regimens also create undesirable and varied opportunistic autoimmune disorders. Among the disorders, autoimmune thrombocytopenia appears fairly frequent. About 3% of non-Hodgkin's lymphoma patients were documented with autoimmune thrombocytopenia following autologous HSCT.[108] In addition, autoimmune hemolytic anemia, Evans' syndrome (immunopancytopenia), hyperthyroidism, colitis, spondylarthropathy, and others have been noted in a recent review.[109]

Allogeneic HSCT from HLA-identical donors has also been used to treat lymphoid malignancies, in particular acute myeloid leukemia. To balance GVHD versus GVL effects, major attention has been given to achieving optimal regimens for lymphoid ablative preconditioning.[110] Periodic reports of autoimmune disorders were associated with preexisting autoimmunity, and a case of relapsing GD was linked to possible stimuli occasioned by chronic GVHD.[111]

HCST in autoimmune diseases

Autologous HSCT is also used to treat autoimmune diseases following various lymphoid ablative regimens. A recent review summarized the results in 155 patients treated for SLE, systemic sclerosis, MS, RA, and Crohn's disease (1996–2006).[95] Of 110 patients treated for the first three diseases, six patients developed secondary autoimmune disorders, including thrombocytopenia, hemolytic anemia, and Factor VIII inhibitor. Comparing patients given ATG versus alemtuzumab, two of 102 versus four of 25 patients, respectively, developed secondary autoimmune disorders. Again, alemtuzumab contributed to immune dysregulation to a greater degree,[96] as seen in MS patients (Monoclonal antibodies targeting T- and/or B-cell compartment).

Allogeneic HSCT has been applied to treatment of autoimmune diseases with occasional reports of secondary autoimmune disorders. Less severe lymphoid ablative regimens are being tested in small trials, and no conclusions can be drawn at this time.[96,110]

Summary

The use of HSCT to treat either lymphoid malignancies or autoimmune diseases can result in opportunistic autoimmune disorders depending on multiple factors, such as genetic predisposition for both donor (autologous and allogeneic) and recipient and the preconditioning procedures. Lymphoid ablation likely includes destruction of Tregs suppressing autoimmune diseases and autoreactive T cells, facilitating the occurrence of autoimmune disorders after HSCT. In treating ongoing autoimmune diseases, the goal is to replace effector cells with a newly emerged repertoire. Using total body irradiation and cyclophosphamide as preconditioning for autologous HSCT, the regeneration of T cells from the thymus was followed for 2 years in seven MS patients, and the new TCR repertoire supported the prolonged reduction in inflammatory activity.[112] It is unknown if the predisposing factors would also regain dominance overtime.

Concluding remarks

As summarized above, there are undoubtedly predisposing factors in patients who developed opportunistic autoimmune disorders despite prescreening. These factors, which are not presently used for prescreening, include family genetics (*HLA* class II and *CTLA-4* genes), gender (which may be aligned with prevalence), prior chemotherapy, and/or immunotherapy, and supportive therapy during immunotherapy. Prior therapy had largely revolved around total body irradiation, cytotoxic drugs, and immunosuppressants, which ablate both Tregs and autoreactive T cells, resulting in opportunistic infections and malignancies while lessoning the risk of autoimmune responses. However, targeting the immune network has made opportunistic autoimmune sequelae seemingly inevitable. Some of these autoimmune sequelae are related to antigen-specific therapy, such as uveitis and vitiligo in melanoma patients receiving peptide vaccine plus anti-CTLA-4, or adoptive transfer of *in vitro*-expanded tumor-infiltrating lymphocytes plus IL-2 administration.[113] When CTLA-4 mAb was used, severe enterocolitis (some likely autoimmune) and autoimmune hypothysitis also ensued.[67,71]

It has been tempting to consider autoimmune responses a promising prognosticator for melanoma treatment with CTLA-4 mAb[67] or IFN-α.[34,35] However, others reported that the presence of anti-retinal antibodies was not associated with prolonged survival.[114] Nor did 74% of anti-CTLA-4-treated patients with mild to severe adverse immune responses show objective improvement.[67] The high-risk low-benefit ratio with anti-CTLA-4 alone and the known association of ATD with IFN-α therapy (Systemic immunomodulators) should raise additional concerns for those contemplating combining the two immunotherapeutic agents in melanoma patients.[115] The simultaneous use of anti-CTLA-4 and IL-2 did not provide a synergistic therapeutic outcome.[116] Moreover, our murine models combining antitumor and autoimmunity under the umbrella of Treg depletion and tumor vaccination clearly suggest mutual stimulation in the presence of autoimmune response and tumor regression (Monoclonal antibodies targeting T- and/or B-cell compartment).

The use of alemtuzumab, a panleukocyte-depleting agent with lasting effects, has demonstrated unintended consequences of targeting the immune network, particularly in treating autoimmune diseases (Monoclonal antibodies targeting T- and/or B-cell compartment). Despite vigorous prescreening, *de novo* ATD and thrombocytopenic purpura were prominent. When coupled with autologous HSCT, the resultant repertoire is difficult to predict.[96] When allogeneic HSCT is used, the need for HLA-matched donors could increase inherent genetic susceptibility, and in addition the generation of GVL or GVH response could provide additional stimuli for development of opportunistic autoimmune disorders. This review has included a number of conditions conducive to development of autoimmune disorders. Any antitumor immunotherapeutic regimen, be it single or combined, should receive serious consideration of potentially severe autoimmune sequelae and be accompanied by sound assessment criteria for early intervention.

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