Evading immune surveillance is one of the common hallmarks of cancer. Herein we describe two major evasion mechanisms in lymphoma, focusing on regulatory T (Treg) cells and C-C chemokine receptor 4 (CCR4) expressed on these cells. First, the tumor cells themselves function as Treg cells, characterized by expression of CCR4, contributing to tumor survival by downregulating host immunity. Second, CCR4 ligands are produced by tumor cells, which attract other CCR4+ Treg cells to the vicinity of the tumor. CCR4+ adult T-cell leukemia/lymphoma is an example of the former phenomenon, and Hodgkin lymphoma of the latter, for which an almost identical immunopathogenesis has been reported in many types of cancer. Awareness of the importance of CCR4 allows the rational design of more effective cancer treatments. Accordingly, we have developed a defucosylated anti-CCR4 mAb, the first therapeutic agent targeting CCR4 to be used clinically for cancer. The therapeutic anti-CCR4 mAb represents a promising treatment method for patients with CCR4+ neoplasms by directly killing the cancer cells, but could also be used as a novel treatment strategy for many types of CCR4+ cancers to overcome the suppressive effect of CCR4+ Treg cells. (Cancer Sci 2011; 102: 44–50)

T he current World Health Organization (WHO) classification of lymphoid neoplasms is based on the use of all pertinent available information such as morphology, immunophenotype, genetic features, and clinical features, and a normal counterpart cell is postulated for each neoplasm.1 B-cell differentiation is relatively well understood, and B-cell neoplasms listed in the current WHO classification correspond well to stages of normal B-cell differentiation. For instance, mantle cell lymphoma, follicular lymphoma, and marginal zone lymphoma correspond to pre-germinal center naïve B cells, germinal center B cells, and post-germinal center marginal zone B cells, respectively, and proliferate at their sites of origin, that is, mantle zone, germinal center, and marginal zone, respectively. In contrast, the classification of T-cell neoplasms, especially mature T-cell neoplasms, which are also called peripheral T-cell lymphomas (PTCL), remains challenging, with approximately 30–50% of cases considered “PTCL unclassifiable” (PTCL-not otherwise specified [NOS]), probably due to complex pathways of T-cell differentiation. Thus, the current PTCL classification may represent a provisional organization of syndromes rather than a list of distinct disease entities.1

Adult T-cell leukemia/lymphoma (ATLL)

Among PTCL, ATLL is a distinct disease entity caused by human T-cell leukemia virus type 1 (HTLV-1). Its diagnosis is confirmed by the monoclonal integration of HTLV-1 into neoplastic T-cells. HTLV-1 is transmitted mainly from mother to infant through breast milk.2 The HTLV-1 receptor is the glucose transporter 1 (GLUT1) protein expressed ubiquitously.3 Thus, HTLV-1 can infect different types of cells in addition to CD4+ T-lymphocytes.4,5 This fact gives rise to the simple question of why HTLV-1 causes malignant transformation only of CD4+ T-lymphocytes, especially CD4+CD25+CD69+ T-lymphocytes.6-7 CCR4 is known to be expressed selectively on regulatory T (Treg) cells and type 2 helper T (Th) cells.8-11

ATLL and Treg cells

CD4+ Th lymphocytes represent a heterogeneous population of cells that play an essential role in adaptive immunity. These cells include effector subsets, such as Th1, Th2, Th17, and follicular helper T (Tfh) cells, which protect against pathogens, and Treg cells, which protect against effector responses to autoantigens and also against over-exuberant responses to exogenous antigens when they may become dangerous for the host. Each effector subset develops through the actions of different transcription factors, such as T-bet for Th1,14 GATA-3 for Th2,15 RORC for Th17,16 and bcl-6 for Th17,17,18 with FOXP3 being the key transcription factor for the development and function of Treg cells.9,19-21 Of the several types of Treg cells, both naturally occurring and induced Treg cells are well characterized; the former are generated in the thymus and the latter from naïve T cells in the periphery (Fig. 1).22,23 Regarding surface phenotypes, Treg cells express CD25 and CCR4, in addition to CD4.8,12 Because tumor cells from most ATLL patients also have this CD4+CD25+CCR4+FOXP3+ phenotype,6,7,12,24-25 based on their phenotypic characteristics, these tumors may originate from CD4+CD25+CCR4+FOXP3+ Treg cells. In addition, we have shown that CCR4+ ATLL cells from a subset of patients do indeed function as Treg cells in an autologous setting.26 This finding provides some insight into why HTLV-1 causes only CD4+CD25+CCR4+ lymphocytes to develop into neoplasia.

Why does HTLV-1 cause malignant transformation of only CD4+CD25+CCR4+ lymphocytes?

As mentioned above, HTLV-1 infects different types of cells, including Treg cells, through GLUT1 in a cell to cell manner. Tax and other viral genes such as HBZ mediate growth promotion in HTLV-1-infected cells, which express viral-associated antigens derived from core, envelope, polymerase, or Tax on their surface. This implies that infected cells must face the host antiviral immune responses.27 Therefore, it is expected that HTLV-1-infected cells should be recognized and eradicated by HTLV-1-specific CTL. Thus, HTLV-1 infected Treg cells may have a survival advantage compared to other types of cells, by
suppressing host immune responses against themselves. As a result, HTLV-1 infected CD4+CD25+CCR4+ Treg cells should preferentially survive, and gradually increase in number. Furthermore, the accumulation of additional crucial genomic and/or epigenomic alterations could cause HTLV-1 infected cells to change into clonally proliferating ATLL cells. Collectively, the natural progression from HTLV-1 infection to ATLL development is illustrated in Figure 2, based on that proposed by Matsuoka et al. (4,5) Silencing HTLV-1 associated antigens, such as Tax, is a generally accepted mechanism to avoid immunity,(27) although it fails to explain selective tumorigenesis caused by HTLV-1 that occurs in CD4+CD25+CCR4+ lymphocytes.

PTCL-NOS

These lymphomas make up a heterogeneous category of mature T-cell lymphomas, which do not correspond to any of the specific defined entities of mature T-cell lymphoma in the current WHO classification.(1) Approximately 30–40% cases of PTCL-NOS are CCR4+,(28–30) and CCR4 expression is an independent and significant unfavorable prognostic factor in these patients.(29) Ohshima et al.(28) also reported that CCR4 expression was an unfavorable prognostic factor for this type of lymphoma. Together with the fact that most ATLL, which belong to the group with the most unfavorable prognosis among PTCL,(31) are positive for CCR4, these findings raise the question why CCR4 expression is an unfavorable prognostic factor for this type of lymphoma. Based on genomic profiling, Nakagawa et al.(30) proposed that PTCL-NOS could be divided into two groups, one with and one without genomic alterations, the former being significantly associated with shorter overall survival time, and frequently showing expression of CCR4 (16 of 26 [61.5%] versus ONE of 22 [4.5%], respectively; P = 0.0001). This study provided new insights into our understanding of CCR4-expressing PTCL, and possible answers to the above question.

Why is CCR4 expression an unfavorable prognostic factor for PTCL-NOS?

Although PTCL-NOS are particularly heterogeneous, the CCR4+ subset may be a distinct disease entity originating from a Treg cell. The finding that CCR4+ PTCL-NOS had significantly higher expression of CD25 compared to CCR4- PTCL-NOS (nine of 13 [69.2%] versus 6 of 31 [19.4%], respectively; P = 0.0038)(29) is consistent with a possible association of CCR4-expressing PTCL with Treg cells. It has been generally accepted that development of cancer, including PTCL, is a multistep process requiring the accumulation of multiple genetic and epigenetic alterations.(32,33) Typically, a T cell acquiring certain initial genetic/epigenetic alterations expresses tumor antigens derived from the resulting mutated genes or modified proteins, and thus would face host immune responses.(34) Probably, only PTCL cells that originate from a Treg cell have adequate time to accumulate the full bevy of genetic/epigenetic alterations required for malignant transformation, because compared to other types of T cells, they are immunologically privileged by their ability to downregulate antitumor immune responses. As a result, PTCL originating from a CCR4 Treg cell may tend to be “PTCL-NOS with genomic alterations”, and have significantly worse prognosis. In contrast, the PTCL that originate from non-Treg cells may tend to be “PTCL-NOS without genomic alterations”. Notably, this hypothetical scenario of “PTCL-NOS with genomic alterations” is extremely similar to that for ATLL, in which most cases are CCR4+ (Fig. 2). (7) In fact, Nakagawa et al. reported that not only clinicopathological features, including overall survival curves, but also the genomic profiles of “PTCL-NOS with genomic alterations”, were very similar to lymphoma-type ATLL.(30,35) This finding supports the hypothesis that “PTCL-NOS with genomic alterations” and ATLL have an identical immunopathogenesis, that is, they both originate from a CCR4 Treg cell.
addition, the significant correlation between the expression levels of CCR4 and FOXP3 mRNA in affected lymph node cells obtained from patients with PTCL-NOS(29) is consistent with a possible association of CCR4+ PTCL-NOS with Treg cells.

Angioimmunoblastic T-cell lymphoma (AITL) and anaplastic lymphoma kinase (ALK)+ anaplastic large cell lymphoma (ALCL)

Recent studies have provided evidence that AITL is a neoplasm that originates from Tfh cells. These cells home to B-cell follicles and induce antibody production by B cells. They express chemokine receptor CXCR5, which allows their migration into the CXCL13-rich B-cell follicles of secondary lymphoid tissues. Expression of CXCR5 as well as BCL6 and CD10 is observed in the majority of AITL cases.(36,37) In addition, the AITL gene signature has been shown to be enriched in genes of normal Tfh cells.(38,39) The cellular origin of AITL from Tfh cells provides a rational model to explain several of the peculiar pathological and biological features inherent to this disease, that is, the intimate association with germinal centers in early disease stages, and the polyclonal hypergammaglobulinemia. Follicular helper T cells are unique regulatory cells that suppress the activation of conventional CD4+ T cells, particularly Th1 cells,(40) a finding that could at least partly explain the immune dysfunctions observed in AITL patients (Fig. 1).

Although the expression of Th17-associated molecules in ALK+ ALCL was noted in the gene expression profiling carried out by Iqbal et al.,(41) it may not fully explain several of the peculiar pathological and biological features of ALK+ ALCL(3) other than its expression of the cytotoxic molecules (Fig. 1).

Epstein–Barr virus (EBV)-associated lymphoproliferative disorders

Epstein–Barr virus has been shown to contribute to the development of several types of mature B-, T-, and natural killer (NK) cell lymphomas. Among B-cell lymphomas, EBV is involved in the pathogenesis of lymphomas such as endemic Burkitt lymphoma, many types of immunodeficiency-associated lymphoproliferative disorders, plasmablastic lymphoma, and EBV+ diffuse large B-cell lymphoma (DLBCL) of the elderly.(41) We focus on the last of these here. Nakayama et al.(44) reported that EBV-infected B cells acquire the ability to produce TARC/CCL17 and MDC/CCL22 through latent membrane protein 1 (LMP1)-mediated activation of nuclear factor κB, and suggested that the production of these factors, which attract CCR4+ Treg cells,
CCR4+ and FOXP3+ cells were abundant among the infiltrating also quite reminiscent of HL.

Antibody-dependent cellular cytotoxicity (ADCC) is one of the most important mechanisms of action of therapeutic mAb;\(^{(49-53)}\) CCR4 as a novel molecular target for immunotherapy of cancer

Recognition of the importance of CCR4 on tumor and Treg cells in cancer immunotherapy, and developed therapeutic chimeric (KM2760) and humanized (KW-0761) anti-CCR4 mAbs.\(^{(49,50)}\) Antibody-dependent cellular cytotoxicity (ADCC) is one of the most important mechanisms of action of therapeutic mAb;\(^{(51-53)}\) however, ADCC depends on the cytotoxic activity of effector cells, such as NK cells and monocytes/macrophages, which are commonly qualitatively suppressed and quantitatively reduced in cancer patients. To overcome this problem, the Fc regions of the therapeutic anti-CCR4 mAbs were defucosylated, to enhance ADCC by increasing antibody binding affinity to Fcγ receptors on effector cells.\(^{(52,59,54)}\) We have reported that robust ADCC of the therapeutic defucosylated anti-CCR4 mAb mediated by autologous effector cells is indeed triggered in some ATLL, PTCL-NOS, and advanced/refractory cutaneous T-cell lymphoma patients \textit{in vitro}.\(^{(55,55)}\) This mAb also showed significant antitumor activity in disseminated and non-disseminated CCR4-positive lymphoma models in SCID mice.\(^{(55-57)}\)

\textbf{Humanized mouse model to evaluate human immunotherapy}

The therapeutic defucosylated anti-CCR4 mAbs (both KM2760 and KW-0761) can induce highly enhanced ADCC activity, but do not mediate complement-dependent cytotoxicity or possess direct antitumor activities.\(^{(49,50)}\) Because there were no suitable small animal models to evaluate human ADCC \textit{in vivo}, due to species incompatibilities, we established a \textit{humanized mouse;\(^{12}\)} in which human immune cells from healthy individuals function as ADCC effector cells against allogeneic tumor cell lines, using NOD/Shi-scid, IL-2R\(^{γc}\)-null (NOG) mice\(^{(58,59)}\) as recipients. In this model, the therapeutic anti-CCR4 mAb showed potent antitumor activity by human ADCC.\(^{(60)}\) Using this humanized mouse model, we had the opportunity to undertake more appropriate preclinical evaluation of many types of mAb-based immunotherapy, although in the initial study, we could not completely exclude non-specific allogeneic immune responses because target and effector cells were obtained from different individuals. To overcome the subsequent problems, we have established a primary human tumor-bearing NOG mouse model, in which autologous human immune cells are engrafted and mediate ADCC but in which endogenous murine cells are unable to mediate ADCC. In that study, we used primary ATLL cells bearing NOG mice.\(^{(61)}\) Figure 4 includes images of both an untreated NOG mouse with primary ATLL cells and a mouse that received the therapeutic anti-CCR4 mAb after inoculation with ATLL cells. The therapeutic anti-CCR4 mAb showed significant antitumor activity against primary ATLL cells by robust ADCC mediated by autologous effector cells from the same patients in NOG mice \textit{in vivo}.\(^{(61)}\) The study was the first to report a mouse model in which a potent antitumor effect of the therapeutic mAb against primary tumor cells is mediated by autologous human immune cells. This approach should make it possible to model the human immune system active in mAb-based immunotherapy \textit{in vivo}, and thus to carry out more appropriate preclinical evaluations of novel therapeutic mAbs.

\textbf{Clinical development of KW-0761}

Based on the promising results of this preclinical work, and as an outcome of the success of this translational research, we have already completed a phase I clinical trial of KW-0761, in a single-agent, dose-escalation, multicenter study for patients with relapsed CCR4+ T-cell leukemia/lymphoma in Japan (http://www.clinicaltrials.gov, Identifier: NCT00355472).\(^{(62)}\) Importantly, this phase I study was the first clinical trial to examine the safety and efficacy of a next-generation defucosylated therapeutic antibody against cancer. Although the number of patients in this trial was small, it is noteworthy that objective responses were achieved in 31% of patients, with 13% complete responses. This is a particularly promising result as the response rate of relapsed patients with ATLL to conventional chemotherapy with...
Treg cells in the vicinity of tumors is a potentially promising strategy for boosting tumor-associated antigen-specific immunity. We showed that our therapeutic anti-CCR4 mAb actually did deplete Treg cells in vivo, and furthermore, also had this activity in vivo in humanized mice. Collectively, these data suggest that therapeutic defucosylated anti-CCR4 mAb may soon become a promising treatment for patients with CCR4+ neoplasms by directly killing the tumor cells. Moreover, in the near future, they could also be used as a novel strategy for treatment of many other types of cancers to overcome the suppressive effect of CCR4+ Treg cells on the host’s immune response to tumor cells.

Conclusions

Cancer is a complex collection of distinct genetic diseases sharing hallmarks such as self-sufficiency in growth signaling, evading apoptosis, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis, evading immune surveillance, and stress phenotype. Of those hallmarks, we focused on “evading immune surveillance”, and here we propose two major evasion mechanisms in lymphoma. First, tumor cells themselves function as Treg cells, contributing to tumor survival in the face of the host immune response; second, ligands for CCR4 are produced by tumor cells and/or the tumor microenvironment, and then attract CCR4-expressing Treg cells to the tumor, where they create a favorable environment for tumor cells to survive despite host immune recognition. CCR4+ ATLL is representative of the former, and HL of the latter, with respect to which, it has been generally accepted that increased Treg cells in the tumor microenvironment play an important role in tumor escape from host immunity in several different types of cancer. Moreover, Treg cells infiltrating the tumor may represent one of the main obstacles to successful tumor immunotherapy. Therefore, depletion of Treg cells in the vicinity of tumors is a potentially promising strategy for boosting tumor-associated antigen-specific immunity. We showed that our therapeutic anti-CCR4 mAb actually did deplete Treg cells in vivo, and furthermore, also had this activity in vivo in humanized mice. Collectively, these data suggest that therapeutic defucosylated anti-CCR4 mAb may soon become a promising treatment for patients with CCR4+ neoplasms by directly killing the tumor cells. Moreover, in the near future, they could also be used as a novel strategy for treatment of many other types of cancers to overcome the suppressive effect of CCR4+ Treg cells on the host’s immune response to tumor cells.
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