THE CHEMOKINE CCL17/TARC AS A BIOMARKER IN HODGKIN LYMPHOMA

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ABSTRACT

Classical Hodgkin lymphoma (cHL) is a lymphoproliferative disorder hallmarked by a distinctive type of neoplastic cells, the Hodgkin and Reed/Sternberg (H/RS) cells. H/RS cells represent only a minor cell population of the total tumour mass and are surrounded by an infiltrate composed of mostly inflammatory cells. This composition results from the reciprocal release of soluble factors, such as cytokines and chemokines and other growth factors. In this context, the chemokine CCL17, also known as thymus and activation-related chemokine (TARC), emerges to have important biological functions, as it is expressed in high amounts by H/RS cells and highly elevated in the serum of cHL patients. CCL17 recruits Th2 cells and regulatory T cells that account for a beneficial microenvironment for H/RS cells. In this review, we summarise the current knowledge on CCL17 in cHL and other leukaemias and lymphomas and provide an outlook into clinical applications of CCL17 as a disease biomarker and as a therapeutic target in cHL.

Keywords: Hodgkin lymphoma, TARC, CCL17, serum biomarker.

Hodgkin Lymphoma: Dependent on Microenvironment

Hodgkin Lymphoma (HL), with an incidence of 3/100,000 per year in the Western world, is a disease which affects mostly young adults. Although HL is regarded as a curable disease as therapy is successful in more than 90% of cases,[1] many patients relapse at later points or suffer from treatment-related secondary malignancies. Two forms of HL exist, nodular lymphocyte predominant HL (which is not linked to elevated CCL17 levels),[2] and classical HL (cHL) which manifests different clinical signs and biology. This review focuses on CCL17 in cHL. The tumour cells in cHL are the mononucleated Hodgkin cells and the multinucleated Reed/Sternberg cells that evolve from Hodgkin cells undergoing endomitosis.[3,4] Interestingly, these Hodgkin and Reed/Sternberg (H/RS) cells constitute only about 1% of the tumour mass and are hugely outnumbered by infiltrating inflammatory cells. These surrounding bystander cells support the tumour and provide survival signals to the H/RS cells, making them highly dependent on their surroundings. The cHL microenvironment includes macrophages, mast cells, plasma cells, B cells, dendritic cells, fibroblasts, neutrophils, eosinophils, and T cells. Of the latter, CD4 T cells often show a tumour-promoting Th2 or regulatory T cell type. For review of HL and its microenvironment see Steidl et al.[5]

CCL17 Recruits Tumour-Promoting Th2 and Treg Cells

Chemokines are small secretory molecules acting as chemoattractants for leukocytes. CCL17, a CC type chemokine, is also commonly addressed as thymus and activation-related chemokine (TARC).[6] CCL17 is encoded in a gene cluster with CX3CL1 and macrophage-derived chemokine (MDC, CCL22) on 16q13 (for review see Colobran et al.[7]). CCL17 is constitutively expressed in the thymus[6] and physiologically secreted by dendritic cells,[8] some endothelial and epithelial cells,[9,10] as well as in some instances by fibroblasts and keratinocytes.[11] CCL17 is expressed by M2a macrophages, a subtype

of macrophages, which can act in a tumour-promoting manner,[12] and inhibits classical (M1) macrophage activation.[13]

CCL17 and CCL22 bind to their receptor CCR4,[14,15] which is characteristically expressed on Th2 cells and regulatory T cells.[16,17] Th2 type immune cells are commonly approved to provide tumour-promoting signals to cancer cells, and regulatory T cells keep reactive immune cells in check, preventing tumour immunosurveillance inter alia by secretion of immunosuppressive cytokines as IL-10 and TGF-β. CCL17 (together with CCL22[18]) recruits these cells into the proximity of H/RS cells in cHL patients. As patients display highly elevated serum levels of these two chemokines, both can be regarded as suitable biomarkers for cHL, with CCL17 potentially being the more potent one as its mean serum values for healthy individuals and patients are set wider apart as compared to CCL22.[19]

Although many studies reveal a purely tumour-beneficial role of CCL17-dependent recruitment of Th2 and regulatory T cells, two groups also report contradicting results. One is that cHL patients with high numbers of Th2 cells in the tumour tissue have a favourable prognosis and many regulatory T cells accompanied by low numbers of Th2 cells account for a poorer prognosis.[20] The second study even showed that many regulatory T cells, together with a low reactive cytotoxic T lymphocyte count, correlate with better prognosis for the patients.[21] Consequently, there is still a need for further experimental/clinical evidence to better understand the tumour-promoting or possibly tumour-opposing roles of Th2 cells and regulatory T cells in cHL.

While this review focuses on the significance of CCL17 in cHL and its role in other haematologic malignancies, it is worth mentioning that CCL17 has also been linked to several other diseases. Among these are skin diseases such as atopic dermatitis (for review see Saeki and Tamaki[22]), allergic diseases as asthma,[23] pulmonary fibrosis,[24] and some solid tumours in which CCL17 might promote metastasis.[25-27]

CCL17 is Highly Elevated in Hodgkin Lymphoma

First hints on CCL17 secretion by H/RS cells were found about 15 years ago, published by the Poppema group.[2,18,28] CCL17-positive H/RS cells have been found in patient tissue and CCL17 serum levels are significantly elevated in cHL patients compared to healthy individuals.[2,18,19,28-32] Moreover, cHL cell lines express and secrete high CCL17 levels.[18,28,32] It was shown that this elevated CCL17 secretion correlates with recruitment of CCR4-positive T cells into the tumour.[33,34]

CCL17 serum levels of cHL patients are dependent on the Ann Arbor stage of disease[35-37] and correlate with tumour burden,[37,38] providing CCL17 as a suitable biomarker for evaluation of response to treatment. Indeed, several studies, one of them published in the *New England Journal of Medicine*, already used CCL17 as a marker for response to therapy.[39-41]

With the largest cHL cohort so far evaluated for CCL17 levels, we established a multivariate model of response to treatment including CCL17 and established risk factors. Following this model, patients with baseline serum CCL17 above a certain threshold have a threefold aggravated risk of therapy failure compared to patients with CCL17 values below that threshold.[36]

In line with these data is Weihrauch's study,[35] which revealed elevated CCL17 levels in 90% of patients. Complete responders exhibit lower CCL17, while cHL patients with progressive disease exhibit higher CCL17 before and after treatment, and high CCL17 levels after therapy are a risk factor for poorer survival.

Plattel et al.,[37] and our own unpublished results, have shown that CCL17 levels drop after treatment in most patients and as early as after one cycle of chemotherapy. In the study performed by Plattel and colleagues,[37] non-responders are the only patients not showing this intense reduction after treatment. Furthermore, this study reveals elevated CCL17 levels in all included recurrent patients at the time of relapse. This implies that monitoring CCL17 serum levels after therapy might be a handy method for early identification of relapse patients. Nevertheless, the impact of CCL17 monitoring to evaluate the freedom of the disease needs further confirmation as Plattel's study investigated a relatively small cohort only.

The Role of CCL17 in Other Leukaemias and Lymphomas

Besides the indisputable significance of CCL17 in cHL, this cytokine also seems to play a role in multiple other leukaemias and lymphomas. While CCL17 attracts a Th2 type microenvironment in cHL, hence acting as an endocrine factor, in several other diseases, the tumour cells secrete CCL17 and express

its receptor CCR4 at the same time, suggesting an autocrine, tumour-promoting mechanism.

Tumour cells with expression of CCL17 and CCR4, can be found in adult T cell leukaemia/lymphoma (ATLL)[31,42-46] as well as in cutaneous T cell lymphoma (CTCL).[47-52] Here, CCR4 expression on the tumour cells often results in skin homing, Treg-like functions of the tumour cells themselves and can be correlated to poor prognosis. At least in CTCL, CCL17 serum levels have prognostic relevance as they correlate with tumour stage and lead to further recruitment of CCR4-positive T cells.[47,48]

It might be noteworthy that at least one report claims CCR4 expression in cHL cell lines as well,[28] while others do not find CCR4 on H/RS cells in tumour tissue.[29] It would seem that the textbook lines have yet to be written, but if there are indeed CCR4-positive H/RS cells, this is likely of functional and therapeutic relevance.

In anaplastic large cell lymphoma (ALCL), which is CD30-positive just like cHL, CCR4 is expressed on tumour cells in some cases, while CCL17 was not found to be elevated, providing CCL17 as a marker for differential diagnosis in morphologically similar tumour types.[2,29,50]

Lastly, only few reports exist on CCL17 secretion by leukaemic cells. In acute and chronic lymphocytic leukaemia (ALL and CLL) some CCL17 production can be measured, which might depend on CD40 ligation.[53-56] In acute myeloid leukaemia (AML), CCL17 levels might even correlate with stage of disease.[57]

CCL17 as a Therapeutic Target in Haematological Malignancies

These data hint to the feasibility of CCL17 and its receptor as a potential therapeutic target in cHL as well as in CCL17 or CCR4-positive lymphomas. There are a few studies demonstrating beneficial effects of such treatment strategies. Ishida et al.[34] were able to inhibit migration of CCR4-positive T cells *in vitro*, potentially impeding the favourable Th2 type microenvironment.

Another study used T cells carrying a chimeric antigen receptor (CAR) specific for the HL tumour antigen CD30. These CAR T cells additionally expressed CCR4 to direct them to the tumour. When they subcutaneously engrafted tumours composed of cHL cell lines in immunocompromised mice, the so engineered T cells exhibited enhanced tumour control.[58]

For immunotherapy of CCR4-expressing ATLL and CTCL cells, CCR4 antibodies are being developed and the first studies have shown promising results.[43,59] In another approach, CCL17 was fused to a toxin and has been tested in mice.[60]

CONCLUSION AND FUTURE REMARKS

Here, we summarise the current knowledge about the biomarker CCL17 in cHL and other leukaemias and lymphomas. In cHL, CCL17 is secreted by H/RS cells, and has important biological functions as it recruits Th2 cells and regulatory T cells that account for a beneficial microenvironment for the tumour cells. CCL17 serum levels are significantly increased in Hodgkin patients, and advanced disease stages exhibit higher CCL17 levels. Adding to that, a multivariate model, taking into consideration pre-treatment CCL17 levels together with established risk factors, showed a three-fold enhanced risk for therapy failure if CCL17 was above a certain threshold. Other studies show rapid normalisation of serum CCL17 immediately after the first cycle of chemotherapy in responding patients; while in non-responders and relapse patients, CCL17 fails to drop. This underlines the impact of CCL17 as a biomarker for therapy outcome in cHL.

Alongside its role as a serum marker, several promising studies have been performed indicating a role for CCL17 (and its receptor CCR4) as an (immuno) therapeutic target. Efforts have been made to inhibit T cell recruitment or to use the CCL17 gradient in cHL patients to direct genetically modified effector T cells into the tumour.

Summarising the overall information on CCL17 in cHL, this chemokine can be regarded as a key player in cHL. Being elevated in about 90% of patients, its levels correlating with stage of disease and predicting if therapy will be successful, makes CCL17 a suitable serum marker that can be analysed quickly and inexpensively by enzyme-linked immunosorbent assay (ELISA). Determination of CCL17 levels should be performed in all cHL patients and be included in clinical studies. Monitoring CCL17 levels throughout and beyond therapy will help to identify non-responders. After treatment completion, measuring CCL17 every couple of months will likely help with the early identification of patients suffering from relapse. All in all, it is beyond question that CCL17 should be kept in mind when thinking about cHL.

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