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In hematological malignancies complex interactions exist between the immune system, microorganisms and malignant cells. On one hand, microorganisms can induce cancer, as illustrated by specific infection-induced lymphoproliferative diseases such as Helicobacter pylori-associated gastric mucosa-associated lymphoid tissue lymphoma. On the other hand, malignant cells create an immunosuppressive environment for their own benefit, but this also results in an increased risk of infections. Disrupted innate immunity contributes to the neoplastic transformation of blood cells by several mechanisms, including the uncontrolled clearance of microbial and autoantigens resulting in chronic immune stimulation and proliferation, chronic inflammation, and defective immune surveillance and anti-cancer immunity. Restoring dysfunction or enhancing responsiveness of the innate immune system might therefore represent a new angle for the prevention and treatment of hematological malignancies, in particular lymphoid malignancies and associated infections. Recently, it has been shown that cells of the innate immune system, such as monocytes/macrophages and natural killer cells, harbor features of immunological memory and display enhanced functionality long-term after stimulation with certain microorganisms and vaccines. These functional changes rely on epigenetic reprogramming and have been termed ‘trained immunity’. In this review the concept of ‘trained immunity’ is discussed in the setting of lymphoid malignancies. Amelioration of infectious complications and hematological disease progression can be envisioned to result from the induction of trained immunity, but future studies are required to prove this exciting new hypothesis.

**Introduction**

In order to combat infections and cancer the human body is equipped with an innate and adaptive immune system. The two systems are highly intertwined and closely collaborate with a bridging role for antigen-presenting cells e.g., dendritic cells. Their differences and commonalities are depicted in Table 1 (for more detailed reviews see references1-3). After birth, the immune system evolves from and is shaped by exposure to foreign antigens, which for the most part come from microbes belonging to the gut microbiota.4 Gradually, an immune armamentarium is build which, by virtue of memory properties, acts increasingly specifically, rapidly and efficiently. Immune memory has in the past been attributed solely to the adaptive immune system, but recently the paradigm has shifted with evidence showing that the innate immune system possesses the capacity for immunological memory, designated ‘trained immunity’.5 This feature is crucial for organisms that
have no adaptive immune response, e.g., plants and invertebrates, but it also exists in humans, where it might be of special benefit in neonates that have yet to develop a mature adaptive immune repertoire. Importantly, ‘training’ of the innate immune system by the use of vaccination, resulting in increased immune activation, has been shown feasible. Hopefully these insights can be exploited in the near future for the design of new treatments for immunodeficiencies, infections and cancer.7

Currently, immunotherapies used for the treatment of hematological malignancies have focused on the adaptive immune system, mostly T and B lymphocyte responses. Examples are numerous and include the use of allogeneic stem cell transplantation (SCT), monoclonal antibodies, immune checkpoint inhibitors and cellular therapies (e.g., adoptive T cell transfer).8-10 However, accumulating evidence has confirmed the significant impact of deregulated interactions between host innate immune cells (e.g., monocytes, dendritic cells, and NK cells) and microbes (e.g., chronic infections and dysbiosis) in the pathogenesis of cancer.11-13 This seems especially true for lymphoid malignancies, where antigenic stimulation by microbes and chronic inflammation drive lymphoproliferation and hence tumor progression.14 Moreover, the immunosuppressive environment that develops in many hematological malignancies, consists of a considerable part of functionally altered innate immune cells, including myeloid-derived suppressor cells (MDSC), that cause immune eva-

sion, progression and dissemination of neoplastic cells.15

Therefore, considering the pivotal role of the innate immune system in the initiation and progression of hematological malignancies, it might prove a valuable target for prevention and treatment. One option would be exploiting the recently discovered feature of innate immune memory which can be induced, for instance, by a Bacillus Calmette-Guérin (BCG) vaccination.1 By enhancing and restoring the function of innate immune cells, clearance of microbial and neoantigens can be achieved and the immunosuppressive tumor microenvironment reversed. In addition, by innate immune training, the infection incidence might be reduced in the high-risk setting of cancer therapy.

In the review herein we summarize the current knowledge on the concept of ‘trained immunity’, and hypothesize ways of extending this concept to the field of lymphoid malignancies. The concept of trained immunity: a novel type of immune memory

For decades the prevailing assumption has been that immunological memory was a feature characterizing only the acquired immune system. However, recent studies have shown that the mammalian innate immune system also exhibits adaptive properties compatible with immunological memory, for which the term ‘trained immunity’ has been proposed.5 On reinfecion or rechall-

enge with microbial ligands, prototypical innate immune cells such as monocytes/macrophages exhibit enhanced functionality with the release of pro-inflammatory cytokines and effector functions e.g., phagocytosis (Figure 1).16,17 The training of monocytes/macrophages is mediat-
ed by the activation of pattern-recognition receptors by microbe-associated molecular patterns (MAMPs)’s from bacteria and fungi; for example dectin-1 by β-glucan, and nucleotide-binding oligomerization domain-containing protein 2 (NOD2) by components of the BCG vaccine.18-19 Several mechanisms are involved in the development of innate immune memory, among which epigenetic histone modifications (e.g., histone methylation and acetylation), and autophagy play a central role. Monocytes are func-
tionally reprogrammed for either enhanced (training) or decreased (tolerance) cytokine production, depending on the type and concentration of MAMPs they encountered.20-22 (Figure 1). The epigenetic reprogramming of monocytes after MAMP stimulation results in functional as well as morphological changes, including cell surface marker modifications such as upregulation of TLR expres-
sion.23,24 Autophagy contributes to the process of ‘trained immunity’ induced by a BCG vaccination, as pharmacological or genetic (autophagy gene polymorphisms) inhibi-
tion blocks the epigenetic programming of monocytes from occurring.25

Natural killer (NK) cells belonging to the innate immune system also display memory functions, mainly during viral infections. Expression of the Ly49H receptor and chemokine receptor CXCR6 seem to be of importance for the memory characteristics of NK cells.26 In addition, epigenetic modifications with changes in promoter methyla-
tion status are a hallmark of adaptive NK cells.27,28 The adaptive immune features of NK cells result in their long-
term activation and protection from infection or reinfection with both herpesviruses and also influenza.24,26,29 NK cell training can also be achieved by BCG vaccination with the induction of non-specific immune memory, as has been shown in healthy volunteers.29

‘Trained immunity’ of innate immune cells provides protection against reinfecion in a direct T/B-cell-independent manner. In addition, a more or less indirect effect on the acquired immune system may also occur as mono-
cytes/macrophages and NK cells influence cells of the adaptive immune system.3 For instance, after BCG vacci-
nation complex cytokine profiles are induced that include elevated T-helper (Th) 1 cytokines like IFNγ and also inter-
leukin (IL)-4 (Th2), IL-17 (Th17), and IL-10.30-32 These responses belong to the adaptive immune responses that facilitate and contribute to infection control and vaccina-
ation efficacy. Important hallmarks of ‘trained immunity’ are the non-specific nature and long duration of the enhanced immune responses. Trained monocytes have been shown to circu-
late in the peripheral blood for up to 3 months after BCG vaccination, and NK responses, e.g., release of IFNγ, are also enhanced for months.33,29 Immune training of both monocytes and NK cells induces protection from infec-
tious agents other than the primary stimulus. In vitro studies demonstrated an increased innate immune response against a plethora of pathogens after BCG vaccination, including Mycobacterium tuberculosis, Candida albicans, and Staphylococcus aureus.34 Moreover, in vivo data exist from large vaccination trials. In low birth weight children from Guinea-Bissau, vaccination with BCG resulted in reduced neonatal mortality, which was the result of a composite effect consisting of reduced neonatal sepsis, respiratory infections and fever.14 However, this protective effect did not result in overall reduced infant mortality, suggesting that it was most pronounced in neonates.4 An alternative explanation, however, might be the fact that the longevity of the training effect is in the order of months and proba-
bly not years, although data on the exact duration are lack-
ing. In Danish cohorts similar results were also achieved...
with BCG and smallpox vaccinations, significantly reducing the risk of hospitalization for most subgroups of infectious diseases, especially respiratory tract infections.\textsuperscript{35,36}

Intriguingly, non-specific protection might go beyond infections, as the BCG vaccination has been shown to be effective in treating carcinomas (e.g., bladder cancer or melanoma).\textsuperscript{37}

Considering the protective effects of ‘trained immunity’ against infections, the concept might be exploited to improve care for cancer patients who suffer from infections. The concept might even be more important in malignancies whose pathogenesis entails exposure to microorganisms (chronic antigenic stimulation) and impaired innate and adaptive immune responses, hence hematological, but in particular, lymphoid malignancies.

**Role for microbes in lymphoid malignancies**

Complex interactions between microorganisms, immune cells and cells exhibiting features of neoplastic transformation eventually determine the development of overt malignant disease. This intriguing interrelatedness of biological events is especially encountered in those circumstances where immune cells themselves are the cells at risk for malignant transformation, thus in the setting of lymphoid malignancies including multiple myeloma (MM), chronic lymphocytic leukemia (CLL) and malignant lymphomas. These diseases constitute ‘exemplary models’ in which the role of ‘trained immunity’ can be explored.

**Infections in patients with lymphoid malignancies**

Patients with cancer suffer from an increased risk for common and opportunistic infections that result from disease intrinsic and anti-cancer therapy-induced immune deficiencies. The severity of these deficiencies and the specific nature of the defects determine which infections can be expected to occur in individual patients. An elaborate review of this topic is beyond the scope of this article and an overview can be found in a recent publication.\textsuperscript{38}

Malignant lymphomas and CLL significantly increase the risk for serious infections.\textsuperscript{39,40} The cause of this increased risk for infections is multifactorial. Early on, the humoral immune abnormalities, resulting from B-lymphocyte dysfunction often accompanied by the emergence of hypogammaglobulinemia, dominate, and infections are caused mainly by viruses and encapsulated bacteria. With the progression of the lymphoid malignancies T cell dysfunction occurs, which is often therapy-related, but not exclusively, as early after diagnosis T cell defects/alterations are already present.\textsuperscript{41,42} Malignant B lymphocytes produce anti-inflammatory cytokines, like IL-10 and TGF-β, and indoleamine 2,3-dioxygenase that skew the balance from a Th1/Th17 towards a regulatory (FOXP3) T cell phenotype.\textsuperscript{43,44} Moreover, tumor cells express immunosuppressive ligands like programmed death-ligand 1 (PD-L1) and PD-L2 that inhibit proliferation and activation of effector memory T cells.\textsuperscript{45} In addition, the recruitment of tumor supporting innate myeloid cells; including nurse-like cells, tolerogenic DCs, tumor-associated macrophages (M2-polarized) and MDSCs; to the tumor micro-environment occurs, which enhance the state of tolerance.\textsuperscript{46-49}

Through these mechanisms, malignant B cells, besides inhibiting anti-tumor immunity, also impair immune responses that are crucial for the control of both common and opportunistic pathogens. Treatment with anti-neo-

**Table 1. Characteristics of innate and adaptive immune responses.**

<table>
<thead>
<tr>
<th>Cellular components</th>
<th>Humoral components</th>
<th>Time to activation</th>
<th>Recognition</th>
<th>Immune memory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphonuclear cells, monocytes, macrophages, dendritic cells, NK cells, innate lymphoid cells</td>
<td>Complement, host defense peptides e.g., defensins, natural antibodies</td>
<td>Early: minutes-hours-days</td>
<td>Non-specific or semi-specific (pattern-recognition receptors)</td>
<td>Epigenetic reprogramming: trained immunity</td>
</tr>
<tr>
<td>T-lymphocytes, B-lymphocytes</td>
<td>Specific antibodies</td>
<td>Late: days-weeks</td>
<td>Highly specific (T-cell receptors and antibodies)</td>
<td>Lasts for weeks to months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gene recombination and clonal expansion</td>
</tr>
</tbody>
</table>

plastic drugs, including purine analogs, and monoclonal antibodies, further impair specific cellular immunity with an increased risk of opportunistic infections.

**Infection-induced lymphoid malignancies**

The role of microorganisms in the pathogenesis of cancer is increasingly appreciated. Several classical pathogens have been implicated in the origin of specific hematologic malignancies. The Epstein-Barr virus is exemplary. It is responsible for the disease infectious mononucleosis, which has also been associated with a diverse range of malignant lymphomas in immunocompetent and immunocompromised patients e.g., Burkitt lymphoma, and post-transplant lymphoproliferative disorder. Other well-known examples are *Helicobacter pylori*, *Borella burgdorferi*, *Coxiella burnetti* and the hepatitis C virus (HCV), which have been associated with marginal zone lymphoma (MZL) and diffuse large B-cell lymphomas (DLBCL).\textsuperscript{40,50} Some of these diseases can initially be treated with antimicrobial agents with a considerable chance for cure, despite the fact that they can be considered monoclonal lymphoproliferative disorders.\textsuperscript{41,51}

Beyond specific pathogens, it has been shown that experiencing common community-acquired viral and bacterial infections early in life increases the risk for developing lymphoid malignancies later in life. Large epidemiological studies have shown clear associations between infections and monoclonal B-cell lymphocytosis (MBL) and CLL, non-Hodgkin lymphoma (NHL), and MM.\textsuperscript{50,52} More recently, the commensal bacterial flora of the gut (microbiota) has also been implicated in lymphomagenesis.\textsuperscript{12}

The expression of restricted immunoglobulin gene repertoires/B-cell receptors (BCR) in CLL and malignant lymphomas underscores a key role of an antigenic drive in the initiation and perpetuation of lymphoproliferation; mainly microbial antigens but also self-antigens released from infected cells.\textsuperscript{53-55} In CLL, over 30% of cases can be grouped together based on the expression of stereotypic BCRs with characteristic complementarity determining region 3 (CDR3) amino acid sequences. As CDR3s are most decisive for the antigen specificity of immunoglobulins (Igs), this strongly suggests that distinctive antigens...
Mechanisms involved in the neoplastic transformation of lymphocytes by microorganisms include: the release of genotoxic metabolites, antigen-driven lymphoproliferation, induction of chronic inflammation, impaired apoptosis, inactivation of the tumor suppressor gene p53, disrupted DNA repair, mitochondrial dysfunction, and oxidative stress. Antigen-driven lymphoproliferation is implicated in the pathogenesis of CLL and NHL, including MZL, mantle cell lymphoma (MCL), follicular lymphoma, and DLBCL (Figure 2). However, B-cells express many other (surface) receptors involved in microbial antigen recognition in addition to the BCR, including CD5 and CD6 (CLL) and pattern recognition receptors like TLR1, TLR2, TLR6, TLR7, TLR9, NOD1 and NOD2 (CLL, NHL). Signaling through these receptors is exploited by the malignant cells for their own survival, precisely as shown for BCR activation. The TLRs expressed by CLL cells, for instance, are functional, as upon stimulation, the nuclear factor-κB signaling pathway becomes activated, protecting CLL cells from spontaneous apoptosis.

Cancer-induced immune-suppression; cancer progression and infections
Many cancers facilitate their preservation and progression by protecting themselves against the host’s immune surveillance armamentarium by inducing an immunosuppressive environment. The acquired immune system seems most affected with pronounced deficits occurring in anti-tumor T cell responses resulting from several interrelated mechanisms, as described above, with an important role for the accumulation of immunosuppressive innate myeloid cells (e.g., MDSC). T cells from CLL patients exhibit deviant T cell subset distributions, and have functional defects, including impaired ability to form immunological synapses, decreased proliferative capacity and an impaired effector function. Targeting these immune checkpoints has been a new approach in the treatment of malignant lymphomas, and is now being explored in large clinical studies.

Also relevant to anti-tumor immune responses are immune cells belonging to the innate immune systems, such as NK cells and monocytes/macrophages. Defects in these immune effectors that contribute to cancer initiation and progression have been increasingly described in lymphoid malignancies. For instance, NK cells found in the cir-
Calculation of CLL patients appear to have several functional defects, including impaired cytotoxic activity, possibly because of the defective expression of the NKG2D co-receptor. Moreover, the monocytic population of CLL patients has an altered composition and monocytes exhibit deregulation of genes that are involved in phagocytosis and inflammation. Higher numbers of non-classical CD14<sup>+</sup>CD16<sup>++</sup> monocytes are present that are known to have immune suppressive features opposed to the classical CD14<sup>++</sup>CD16<sup>–</sup> counterparts. Intriguingly, monocytes from CLL patients exhibit the primary features of ‘endotoxin tolerance’, including low cytokine production, high phagocytic activity and impaired antigen presentation. This refractory state of these cells prohibits sufficient inflammatory responses to occur after pathogens and cancer cells (‘tumor tolerance’). This introduces a new mechanism of innate immune failure that contributes to the susceptibility of CLL patients to infections and tumor progression.

**Trained immunity in hematological malignancies**

*Evidence for ‘trained immunity’ in cancer therapy*

Training the innate immune system seems feasible and effective, as for decades BCG vaccination has been successfully applied in the treatment of urothelial cell carcinomas and melanomas. Data from large epidemiological studies and vaccination trials reveal circumstantial evidence for a similar potential in hematological malignancies. In a previous Danish case-cohort study it was shown that BCG vaccination during infancy significantly reduces the risk of developing lymphoma’s (HR 0.49 (95% CI: 0.26–0.93)). Mechanisms proposed, attempting to explain this beneficial result, included the stimulating effect of vaccination on immune surveillance of cancer and the decreased incidence of infectious diseases involved in lymphoma pathogenesis. Older studies have tested the concept of the induction of anti-tumor immunity in patients with AML by vaccination. However, these studies were small, and although some small benefits were shown, these data preclude drawing definite conclusions. At least BCG vaccination in these patients did not result in untoward complications.

The use of β-glucan as an immune adjuvant in the treatment of solid and hematological malignancies has evoked considerable interest for many years, as the MAMP shows promising activity both *in vitro* and *in vivo*. The anti-tumor...
activity directly relates to signaling through dectin-1, suggesting that the innate and acquired immunity elicited by β-glucan could be of therapeutic value. In a small phase I/II study, twenty patients with advanced malignancies who were receiving chemotherapy were additionally treated with a β-(1,3)/(1,6)-D-glucan preparation. Preliminary results showed that β-glucan was well tolerated in these patients and suggested a beneficial effect on hematopoiesis. Moreover, one patient with a chemotherapy-refractory malignant lymphoma achieved a partial response.

Mechanisms of action

The concept of ‘trained immunity’ can be explored as a new modality of cancer prevention and therapy, as well as in infection control. The concept seems appropriate to consider in the setting of lymphoid malignancies, and three major mechanisms can be envisioned to contribute to the effects of innate immune enhancement in this setting (Figure 3).

1. Decreasing antigen-driven lymphoproliferation:

Many data support the hypothesis that malignant B cells, found in CLL and NHL e.g., MZL and MCL, resemble antigen-activated B cells and that ongoing antigen-induced modulation of cell responses occur in the context of antigen recognition by the BCR and affiliated receptors (Figure 2). Inhibiting this signaling cascade has already proven to be of clinical use with the introduction of potent BCR inhibitors, e.g., ibrutinib, and therefore eradicating B-cell stimulating antigens and infectious antigens and autoantigens may also alter the course of lymphoproliferative diseases. This might be achieved by ‘trained immunity’, as monocytes and macrophages can be activated to prevent many infections that have been implicated in the pathogenesis of lymphoma. Since monocytes and macrophages are also pivotal in apoptotic cell clearance, training of these cells might contribute to autoantigen eradication.

2. Reversing immune tolerance:

Considering the contribution of monocytes that exhibit ‘endotoxin and tumor tolerance’ to tumor progression and infection risk, the reversal of their refractory state by ‘trained immunity’ might prove clinically beneficial. An increased tolerogenic state of monocytes and macrophages has clearly been implicated in lymphoid malignancies. Nevertheless, whether vaccination with BCG or β-glucan can restore the balance and skew tumor associated macrophages from the M2 towards a more M1 phenotype with beneficial anti-tumor characteristics,
Training of NK cells by exposure to β-glucan, resulting in the loss of its immunosuppressive phenotype and gain of enhanced antigen presenting capacities. Hence, the training of NK cells by exposure to BCG or CMV antigens and/or cytokines may prove to be a novel approach for the optimization of NK cell based immunotherapies.

Conclusion

Epigenetic reprogramming of innate immune cells results in enhanced non-specific immunity and immune memory. ‘Training’ of the innate immune system is a novel concept in immunology and infectious disease that may be exploited in hematological malignancies, especially lymphoid malignancies, where antigen-driven lymphoproliferation and immune impairments are the hallmarks of the disease. Amelioration of infectious complications and cancer progression can be envisioned as goals of ‘trained immunity’ in cancer therapy. Acknowledging the current limited data on the effects of ‘trained immunity’, and the hypothetical status, thus far, of the concept in the setting of clinical hematology, further studies are mandatory.

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