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The Role of Natural Killer Cells in Autoimmunity

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Introduction

The role of Natural killer cells in autoimmunity

Autoimmune diseases are characterized by the selective attack or destruction of a single cell type or tissue by auto-reactive cells of the immune system. The cause of attack of cells is due to loss of self-tolerance of cells like T and B cells which are part of the adaptive immune system. These cells are the major cause of pathology in autoimmunity disorders like type-1 diabetes and rheumatoid arthritis [1,2]. Most autoimmune disorders are characterized by sites of chronic inflammation [3,4]. In addition to T and B cells, other immune cells like natural killer (NK) cells are also present in the target organs [5-7]. NK cells are lymphocytes of the innate immune system, with potent effector functions. NK cells can directly lyse target cells, produce cytokines and interact with other immune cells like DC and T cells [8-10]. These functions of NK cells can contribute to autoimmunity either by protecting against or enhancing autoimmunity. NK cells could target and kill auto-reactive T-cells, but NK cells could also recruit auto-reactive T-cells to sites of infection [1,11]. The levels of circulating NK cells are lower in patients with autoimmune disorders and have a reduced function [12-15]. This reduced function might contribute to autoimmune disorders, by preventing NK cells to functions as that these cells normally would. The reduced function of NK cells, lower numbers and that NK cells are found in the target organs, suggests that NK cells may contribute to autoimmune disorders. The nature of NK cell function in autoimmunity is not clear; NK cells can protect against or enhance autoimmunity. Thus what is the nature of NK cell function in autoimmune diseases and could NK cell function be manipulated to prevent or treat autoimmune diseases?

The aim of this review is to discuss the role of NK cells in autoimmune diseases and their use as potential drug targets. The first focus will be on NK cells function under healthy conditions and how self-tolerance is maintained. Next, the role of NK cells in the development and pathology of autoimmune disorders will be discussed using two autoimmunity model systems: type-1 diabetes (T1D) and rheumatoid arthritis (RA). Finally possible therapies for autoimmune diseases using NK cells as targets will be proposed.

Natural killer cell function and education

Effector mechanisms of natural killer cells

Natural killer (NK) cells are lymphocytes of the innate immune system with potent effector functions. These cells are important in the attack of virus-infected cells, especially in the early stages of infection and in tumour surveillance [8-10]. One important effector function of NK cell is their ability to directly kill target cells. This cellular toxicity is mediated through the release of granules containing granzyme B and perforin. Next to release of granules NK cells can also induce apoptosis via the Fas-FasL signal transduction pathway [16]. A third effector function of NK cells is the production of cytokines in response to infection. The main cytokines that NK cells produce are interferon-γ (IFN-γ), tumour necrosis factor (TNF) and transforming growth factor β (TGF- β) [17]. These cytokines have an immune regulatory function like the initiation of the adaptive immune response [18,19]. NK cells can also interact with other cells of the innate and adaptive immune system, like macrophages, dendritic cells, T and B cells [20-23].

Natural killer cell receptors

NK cell function is regulated via receptors that are expressed on the cell surface, the NK cell receptors. Unlike T cells which express one single variable receptor, NK cells express a multitude of germline encoded receptors [34]. NK cell receptors do not arise through gene rearrangement and recombination as is seen in T cell receptors [34]. This indicates that NKRs are not as variable as T cell receptors. Nevertheless, NK cells are very capable of discrimination between self, non-self, diseased and healthy cells.

NK cell receptors are either inhibitory or activating (*table 1*) [35]. Different activating and inhibitory receptors are expressed on NK cells and multiple receptors are expressed on one cell at the same time. Several families of NK cell receptors exist (*table 1*). The family of inhibitory receptors contains the killer cell immunoglobulin-like receptors (KIR) or Ig-like receptors, the C type lectin receptors and leukocyte inhibitory receptors. Activating receptors are the Ig like receptors, the natural cytotoxicity receptors and the C type lectin receptors like the mouse specific Ly49 family of receptors (as reviewed in [35]).

Inhibitory receptors are either major, Histocompatibility Complex (MHC) class I specific or non-MHC class I specific. Examples of MHC class I specific inhibitory receptors are CD158, CD94-NKG2A and LIR1 and examples of non-MHC specific inhibitory receptors are MAFA and LAIR-1 (*table 1*)[36,37]. All inhibitory receptors contain a intracellular signalling motif called the ITIM (Immune-receptor tyrosine-based inhibitory motif), which is

Table 1. *Natural killer cell receptors.* A brief overview of natural killer cell receptor families and some of their known family members and their ligands.

| Inhibitory receptors	Receptor	Ligand	Reference
Ig-like receptors	CD158	HLA-C	[24]
C type lectin receptors	CD94-NKG2A	HLA-E	[25]
	MAFA	E-, N-, R-cadherin	[26]
Leukocyte inhibitory receptors	LIR1	HLA-class I	[27]
	LAIR-1	Collagen	[28]
Activating receptors			
Ig-like receptors	2B4	CD48	[29]
C type lectin receptors	Ly49H	MCMV-m157 (viral protein)	[30]
	CD94-NKG2C	HLA-E	[25]
	NKG2D	Mouse Rae-1	[31]
Natural cytotoxicity receptors	NKp46	Influenza Hemagglutin	[32]
	NKp44	Influenza Hemagglutin	[33]
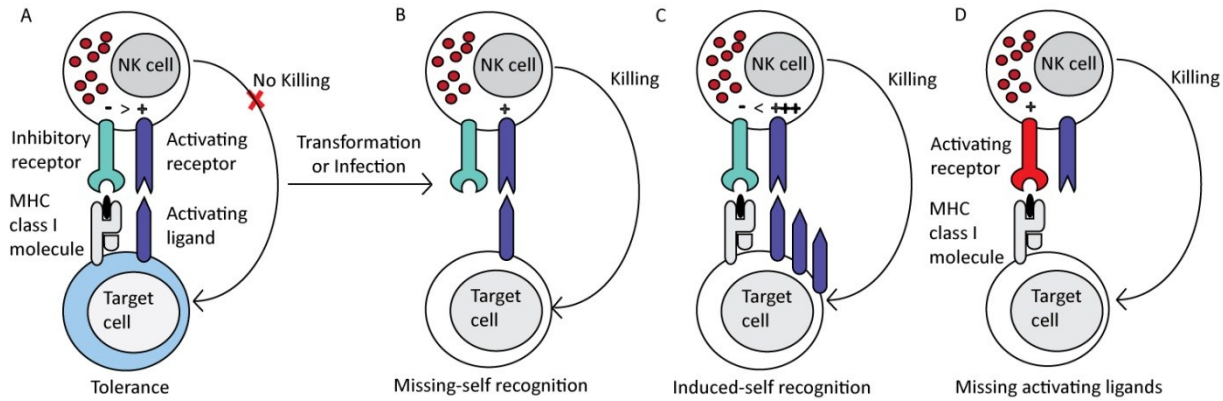
Figure 1. *NK cell activation through missing-self-recognition and induced-self-recognition*. A) In NK the balance between the received activating and inhibitory signals results in tolerance. B) When a target cell loses its MHC class I expression due to infection of transformation, the NK cells gets activated due to the loss of an inhibitory signal. The NK targeted killing of cells lacking MHC class I expression is known as "missing-self recognition". C) Transformation or infection can induce increased expression of activating ligands on a target cell. This overexpression causes for a stronger activating signal. This activating signal can overcome the constitutive inhibition signal generated by the MHC class I, resulting in the activating of the NK cell, this process is known as induced-self recognition. D) Due to infection or transformation a cell might lose all of its activating ligands, preventing cross-liking of activating receptors. In this case the NK cell can still be activated through cross-linking of MHC class I specific activation receptors. Adapted from Raulet *et al.,* [38].

characterized by the consensus sequence Ile/Va/Leu/Ser-X-Tyr-X-X-Leu/Val (X is any amino acid). When an inhibitory receptor is cross-linked by its ligand the tyrosine residues in the ITIM are phosphorylated, resulting in phosphatase recruitment [39]. This recruitment of phosphatases impairs activating signals, resulting in inhibitory signalling.

Activating NK cell receptors are either MHC class I specific (CD94-NKG2C) or non-MHC class I specific (2B4, Ly49H, NKG2D, NKp46 and NKp44) (*see table 1*). Most ligands of non-MHC class I specific receptors are ligands that are expressed on infected, stressed or tumour cells. An example of this is the activating Ly49H receptor, Ly49H is cross-linked by a viral protein, m157 of the mouse cytomegalovirus (MCMV)[30]. For activating signals from to be transduced, the activating receptor needs to associate with an ITAM (Immunodominant tyrosine based activation motif)-containing adaptor protein like Dap12 and CD3ζ [39]. The ITAM is characterized by the consensus sequence Tyr-X-X-ILe/Leu-$X_{(6-8)}$-Tyr-X-X-Ile/Leu (X is any amino acid). Receptor association with an ITAM leads to the activating of the Src family of protein tyrosine kinases. These kinases can phosphorylate both tyrosine residues within the ITAM, which leads to the recruitment and activation of Syk kinases. This activation will than trigger a signalling cascade resulting in the activation of the NK cell [39].

Through the cross-linking of activating and/or inhibitory NK cell receptors, NK cells are activated or inhibited [35]. One of the earliest discovered mechanisms of NK cell activation is loss of expression of self-MHC. It was found that cells lacking MHC class I were more efficiently targeted and killed by NK cells than cells expressing MHC-I, this is called the missing-self hypothesis [40]. When a NK cell encounters a healthy, non-diseased cell, ligands on the cell surface will cross-link activating NK cell receptors, resulting in NK cell activation (*figure 1A*). Crosslinking of inhibitory receptors by MHC I proteins that are expressed on the same cell results in NK cell an inhibition. In a situation when the activating signals are as strong as the inhibitory signals, the NK cells are not activated and thus are tolerant (*figure 1A*) [41]. Upon infection or transformation cells may lose their surface expression of MHC class I (*figure 1B*). In this situation the activating receptor is cross-linked by its ligand, resulting in an activating signal. The inhibitory NK cell receptor cannot be cross-liked since its ligand is absent. The activating signal is now not inhibited by the inhibitory signal, resulting in activation of the NK cell termed missing-self recognition (*figure 1B*)[42]. As an example of this is that certain viral proteins can cause for the down regulation of MHC class I molecules [43]. The opposite may also occur, a cell which expresses MHC class I proteins and through infection or transformation has lost expression of ligands which can cross-link activating

receptors. (*figure 1D*). In this situation the NK cells will not receive any activating signals. The target cell still expresses MHC class I proteins which can cross-link MHC class I inhibitory receptors, and these MHC class I proteins can also cross-link MHC class I activating receptors (*table 1*). MHC class I activating receptors cross-linked by MHC class I proteins can thus still activate the NK cell [44]. Transformation or infection can also induce overexpression of ligands which are able to cross-link activating receptors (*figure 1C*). These cells still express MHC class I proteins, and inhibitory MHC class I receptors are still cross-linked. The inhibitory signal however, is overcome by the activating signals from the cross-linked activating receptors resulting in activation of the NK cell (*figure 1C*). This is called induced-self activation, because the target cell still expresses its self-specific MHC class I [38,42]. The activation of NK is determined through a process which integrates all signals of cross-linked inhibitory and activation receptors. The balance between these signals determines NK cell function.

Self-tolerance in natural killer cells

When there is a balance between activating and inhibiting signals, NK cells become tolerant and do not kill target cells. Although MHC class I specific inhibitory receptors are essential for self-tolerance, not all NK cells express these receptors, yet these NK cells are self-tolerant [45]. This can be explained by the fact that in addition to MHC class I specific inhibitory receptors also other inhibitory receptors specific for self-proteins exist (*table 1*)[46]. NK cells that lack MHC class I specific inhibitory receptors are phenotypically different from NK cells expressing MHC class I specific inhibitory receptors. These missing-self NK cells are non-functional and hyporesponsive to activating signals [45]. MHC class I specific inhibitory receptors not only cause for inhibition of effector function, these receptors also cause for the functional maturation of NK cells, a process known as licensing[47]. Licensing occurs through the recognition of MHC class I by an MHC class I specific inhibitory NK receptor. Similar to the inhibitory effector function of the MHC class I inhibitory receptor the ITIM is essential for licensing. During licensing there is a different, not yet fully identified, signal transduction pathway used than that is used for inhibition [47]. NK cells missing self-recognition thus lack the licensing signal provided via the cross linking of MHC class I specific inhibitory receptors, leaving these cells to be immature and hyporesponsive. Licensing by MHC class I protein mediated cross-linking of inhibitory receptors thus gives rise to two types of NK cells. (1) Licensed, these NK cells express MHC class I specific inhibitory receptors, are functionally mature and self-tolerant. (2) Unlicensed, these NK cells lacking self-recognition and are self-tolerant because they remain immature [47,48].

Two models exist that explain how tolerance occurs through licensing; the "arming" and the "disarming" model. The "arming" or "activating receptor" model states that NK cells are initially unresponsive. Through cross-linking of the MHC class I specific inhibitory receptor functional maturation is induced in the NK cell (*figure 2A*)[47]. Also through the inhibitory signals from the MHC class I receptor, the licensed cell is self-tolerant. The inhibitory signal inhibits activating signals and in thus prevents auto-reactivity (*figure 1*). NK cells missing self-MHC class I recognition do not acquire functional maturity, since these NK cells lack the essential inhibitory MHC class I signal. The "disarming" or inhibitory receptor model sates that NK cells are initially responsive. In the absence of the MHC class I inhibitory receptor mediated licensing signal the NK cell becomes angeric. In the "disarming" model NK cells do not need recognition of self MHC-I by inhibitory receptors to become licensed, but NK cells need it to remain mature. This "disarming" model proposes that the inhibitory MHC class I signal needs to be balanced by an activating signal in order for the NK cell to be licensed (*see figure 2B*). At the same time this balance causes for the NK cell to be self-tolerant (*see figure 1A*)[44]. NK cells lacking self-MHC class I recognition become self-tolerant through "disarming". These cells cannot counter activating signals from target cells, since they lack the inhibitory signal from MHC class I receptors. These activating signals can constitutively signal to the NK cells, since no inhibitory signal is present to counter. This causes for the NK cell to become anergic or hyporesponsive for these activating signals. Meaning the NK cell cannot become activated by these signals and has been "disarmed" (*figure 2B*). These models show different explanations to how NK cells become functionally mature and self-tolerant. For both models evidence has been presented (as reviewed In [49]).
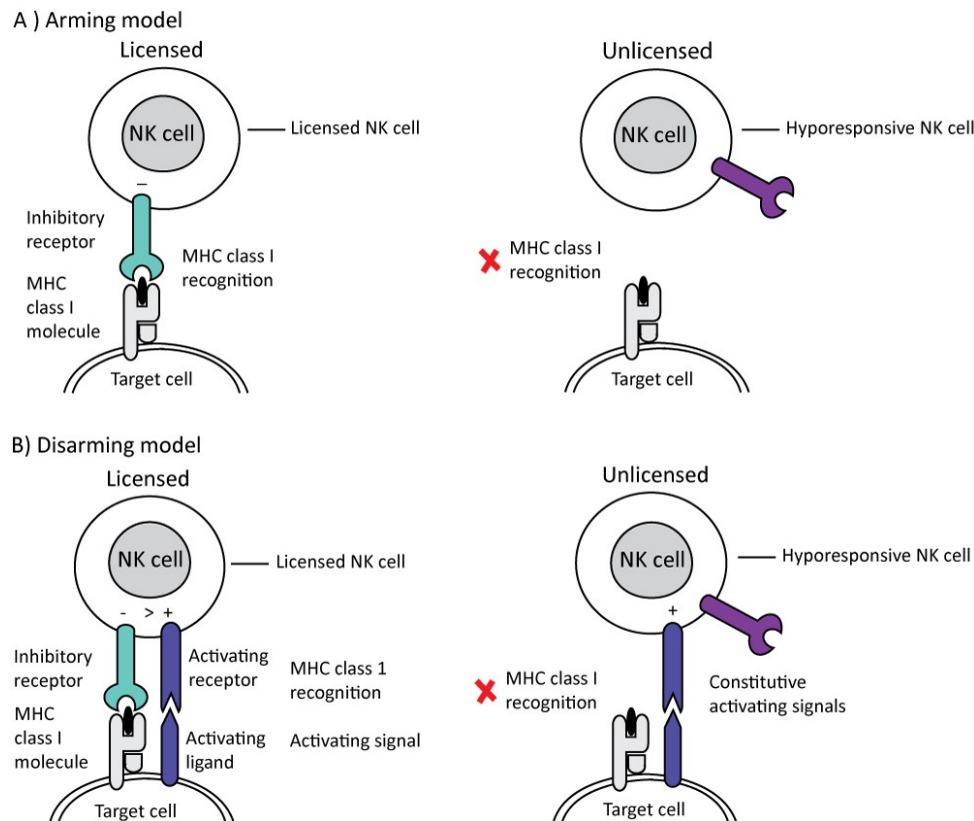
Figure 2. *Licensing in NK cells, the arming and disarming model*. A) The arming model, NK cells are initially unresponsive. NK cells are licensed through recognition of self-MHC class I via an inhibitory receptor; this will induce functional maturation resulting in a licensed cell. NK cells lacking self-MHC class I recognition will not acquire functional maturation and remain unlicensed and hyporesponsive. B) The disarming model, NK cells are initially responsive. NK cells lacking self-recognition will be rendered hyporesponsive through constitutive activating signals. These activating signals can be countered through inhibitory signals from self- recognizing MHC class I receptors, this causes for a balance of signals in NK cells and thus preventing the induction of hyporesponsiveness. Adapted from Raulet *et al.,* [38].

In both models it is stated that unlicensed, missing-self recognition, NK cells are hyporesponsive to activating signals. This is not entirely correct; in response to high doses of plate-bound activating anti-bodies unlicensed NK cells can produce cytokines like IFN-γ [47]. The IFN-γ production was still much lower than seen in licensed NK cells given the same dose of plate-bound activating anti-bodies [47]. This data indicates that licensing is only one of the mechanisms to acquire self-tolerance, since unlicensed NK cells can still be activated.

Other mechanisms may prevent auto-reactivity in NK cells. There are many other inhibitory receptors non-specific for MHC class I present on NK cells. These are usually specific for other widely expressed host proteins and can inhibit NK cell activation and thus auto-reactivity [46]. Also most NK cell activating receptors need co-stimulatory receptors for activation and these receptors are mostly only expressed when inflammation is present in the host. Also other immune cells can regulate NK cell function. Dendritic cells can inhibit NK cells and regulatory T cells can specifically inhibit NK cell function of auto-reactive NK cells [20,38]. However the most important protection against autoimmunity are pro-inflammatory cytokines. For full effective NK cell function not only licensing is needed but also cytokine stimulation. Licensed NK without cytokines stimulation are significantly less functional than licensed NK cells stimulated with cytokines.

The contribution of natural killer cells to autoimmune diseases

NK cells can protect against and enhance autoimmune diseases

It is clear that NK cells have many mechanisms by which auto-reactivity is prevented. Nevertheless NK cells are associated with many autoimmune disorders, indicating that perhaps NK cell tolerance can be broken (as reviewed in[50-52]). It is known that unlicensed, thus possible auto-reactive, NK cells can still be activated *in vitro* by stimulation of high doses of plate bound antigen [47]. Also *in vivo* unlicensed NK cells can be activated as is seen in a CMV infection. Here NK cells lacking self-recognition are strongly represented in the immune response against the infection, suggesting that unlicensed NK cells can become activated in response to infection [45]. Inflammatory cytokines are known to be able to active NK cells during inflammation [8,47]. *In vitro* as well as *in vivo* cytokines are able to activate unlicensed NK cells; these activated unlicensed NK cells have potent effector functions and be possibly auto-reactive [48]. Not only the auto-reactive NK cells can contribute to autoimmunity also non-auto-reactive NK cells can in theory contribute to autoimmunity.

NK cells can contribute to autoimmune disease in different ways, they can have a protective against or enhance autoimmune disease (*figure 3*) [(51)]. Enhancement of autoimmune disease by NK cells can be mediated through cytotoxicity. Auto-reactive NK cells can cause the destruction of cells in the target organ (*Figure 3*). Also the production of cytokines by NK cells can cause for enhancement of autoimmune diseases. Cytokines can activate possible auto-reactive T and B cells [19]. NK cell cytotoxicity and cytokine production may also play a protective role in autoimmune disease (*figure 3*). Cytotoxicity can also kill auto-reactive immune cells and cytokine production can activate regulatory T cells, which can help control the autoimmune disease (*figure 3*) [18,23]. NK cells thus can have a dual function in autoimmunity, either protect against or enhance autoimmune disease. To illustrate these functions of NK cells in autoimmunity and development to autoimmunity, the NK cell function in two model systems for autoimmunity will be discussed. The two model systems that will be discussed are type-1 diabetes (T1D) and rheumatoid arthritis (RA).
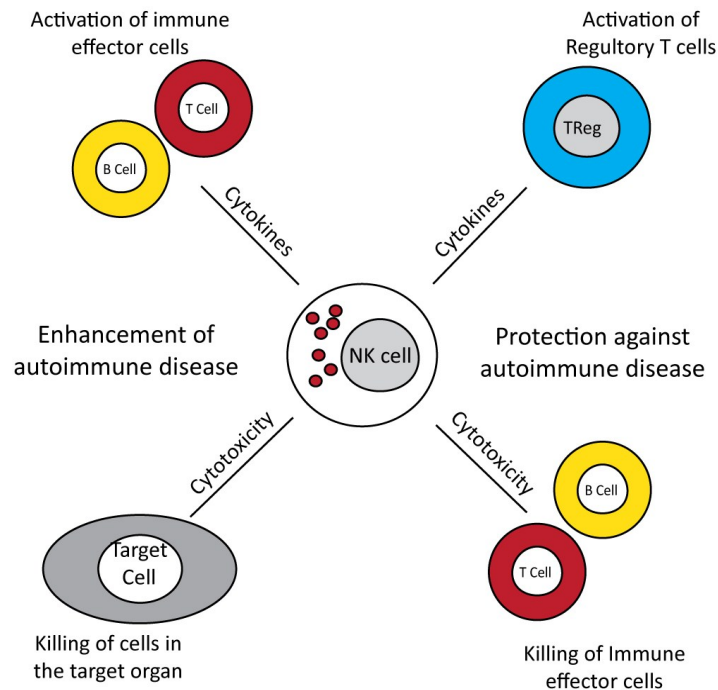

Figure 3. *Possible NK cells functions which can contribute to autoimmune diseases*. Possible functions which can enhance autoimmune diseases are the destruction of cells in the target organ via NK cytotoxicity and the activation of possible auto-reactive immune effectors cells as T and B cells. Possible functions of NK cells which can provide protection against autoimmune diseases are the killing of auto-reactive immune effector cells as T and B cells. Also the activation of regulatory T cells by cytokines produced by NK cells can provide protection from autoimmune diseases.

The role of natural killer cells in Type-1 Diabetes (T1D)

Type-1 diabetes (T1D) or insulin dependent diabetes mellitus is a multifactorial autoimmune disorder. The disease is characterized by the destruction of the pancreatic insulin producing β-cells by auto-reactive T cells, resulting in the loss of glucose homeostasis [53]. The main model used to study T1D is the non-obese diabetic (NOD) mice model. These mice spontaneously develop diabetes after a certain time of insulitis, inflammation of pancreatic islets [54,55]. This is similar to the development of T1D in humans; inflammation of the pancreas precedes the actual destruction of β-cells by auto-reactive T-cells [53]. Despite that T1D is seen as a predominantly T cell mediated disease, NK cells have been found in the pancreatic islets before T cell infiltration and several studies show a decreased or impaired function of NK cells in T1D, [1,56-58]. Furthermore depletion of NK cells in NOD mice resulted in inhibition of T1D development [59]. These studies indicate a role for NK cells in the development of T1D.

The onset of T1D is characterised by inflammation of the pancreas, resulting in an influx of immune cells. In the NOD mice model the influx of NK cells was associated with the progression of insulitis to T1D [60,61]. This has also been reported in the development of human T1D [62]. The infiltrating NK cells may contribute to T1D development in different ways (*figure 3*). One of these possible functions of NK cells contributing to autoimmunity is the killing of cells in the target organ. It has been shown that activated NK cells can target and directly kill pancreatic β-cells [60,63,64]. Something on these pancreatic β-cells thus causes for NK cells to break their self-tolerance and kill the pancreatic β-cell (*figure 1*). Recently the molecular mechanism of this was discovered [1]. The activating NK cell receptor NKp46 is cross-linked by an unknown ligand on pancreatic β-cells. The cross-linking of NKp46 results in NK cell degranulation and killing of the pancreatic β-cell [1]. This unknown ligand expressed by the pancreatic β-cells probably resembles a foreign antigen and due to molecular mimicry NKp46 is cross-linked by this ligand resulting in NK cell activation [1]. Pancreatic β-cells express the ligand for NKp46 also under healthy, non-diseased conditions, but here diabetes does not develop. This suggests that NK cells are most likely not present in the pancreas under healthy, non-diseased circumstances. This is indeed the case, NK cells are only seen in the pancreas during infection or inflammation [1]. Under normal circumstances NK cells do not come in contact with pancreatic β-cells. However when inflammation is present in the pancreas, caused by for example a virus, NK cells will be targeted to the pancreases. These NK cells can encounter the ligand expressed by pancreatic β-cells, and the cross-linking of the NKp46 receptor by this ligand results in the lysis of the pancreatic β-cells. In addition NK cells are thought to promote T cell infiltration in the pancreas via the production of cytokines. Also possible auto-reactive T cells can be recruited and these cells can than target pancreatic β-cells [56].

The previous data shows that NK cells enhance T1D. Depletion of NK cells or removal of the NKp46 receptor in NOD mice during the insulitis or pre-diabetic stage of TID thus prevented pancreatic β-cell destruction and significantly inhibited T1D development [1,59]. Other reports show a protective function of NK cells in TID. In NOD mice and in humans NK cells have a diminished cytokine production and a decreased cytotoxic activity in T1D when compared to controls. These impaired functions have been proposed to contribute to disease pathology [56,57,65]. Two possible explanations for the hyporesponsiveness of NK cells in T1D exist. Firstly, due to the chronic inflammatory environment in the pancreas the NK cells are overstimulated, and this chromic stimulation may lead to hyporesponsiveness (*figure 2B*) [66]. Alternatively, NK cells are actively prevented from mediating effector functions by other immune cells, like regulatory T cells [67].

When in NOD mice NK cells were activated during pre-diabetic stages this prevented diabetes development, suggesting a protective role for NK cells in T1D [65,68,69]. These results come from NOD mice models which were treated with Freund's adjuvant (CFA), an immune booster, resulting in NK cell activation and increased IFN-γ production. The increased IFN-γ production is suggested to lead to the decrease of cytotoxic T cells and prevention of T1D development [68,70,71]. In humans impaired function of NK cells has only been shown in individuals with long standing T1D [72,73]. These differences could perhaps be explained due to that NOD mice have been especially bread to spontaneously develop diabetes.

Impaired NK cell function might contribute to the development of insulitis, since impaired NK cells are not as efficient in preventing and combating infections. This insulitis thus in turn will result in NK cell mediated pancreatic β-cell destruction and development to diabetes. Thus immune stimulation of NK cells in pre-diabetic NOD mice may prevent T1D development. In older NOD mice however, immunostimulation via CFA results in deleterious effects, which is in more conformity with the results seen in humans and the NKp46 receptor[71]. Thus NK cells initially cause for the destruction of β cells and the invasion of T cells into the pancreas, but later on become hyporesponsive due to exhaustion or due to regulation by other immune cells [56].

These results show potential for NK cell based therapies for the prevention and inhibition of T1D development. Preventing activation of NK cells through NKp46 can be done via administering blocking anti-bodies against NKp46. These could quickly block NK cell activation, but as long as there is an inflammation or infection of the pancreas these blocking anti-bodies should be administered [1]. Another way is to immunize individuals against NKp46, preventing NK cell activation through this receptor via the production anti-bodies. However other functions of the NKp46 receptor could then be disturbed, like NKp46 mediated activity against viruses and tumour surveillance [1]. The major drawback of these possible therapies is that these can only be effective in the developmental stages of T1D. Since NK cells eventually become hyporesponsive and these therapies do not inhibit auto-reactive T cell function.

The role of NK cells in Rheumatoid arthritis (RA)

The second model for autoimmunity is rheumatoid arthritis (RA). This is an autoimmune disease characterised by chronic inflammation of the joints. The inflammation is present in the synovium (or synovial membrane) and is characterized by infiltration of NK cells, macrophages, T and B cells, and the continuous excessive production of pro-inflammatory cytokines. During disease progression the inflammation, cytokine production and infiltration of immune cells in the synovium will lead to progressive destruction of bone and cartilage [74]. RA is considered a T- cell mediated disease. Especially a subset of T helper cells, Th17 cells, which are present in the synovium are important in disease pathogenesis[75]. In the collagen induced arthritis mouse model of RA, the inhibition of Th17 cells through interleukin 6, arrests disease development, especially inflammation in the synovium[76]. This suggests that Th17 cells are important effectors in the inflammatory response in RA [75].

NK cells may contribute to RA pathogenesis through cytokine production and cytotoxicity (*figure 3*). These NK cell functions may enhance or protect from RA disease pathogenesis [77]. Enhancement of autoimmunity by NK cells could be mediated in a similar manner as is seen with T1D, that NK cell cytotoxicity is the cause for tissue damage. However the cytotoxic activity of NK cells in the synovium of RA patients was lower when compared to NK cells of healthy individuals. This decreased function may prevent the killing of auto-reactive cells by NK cells via cytotoxicity and thus contribute to disease pathogenesis [78]. Interestingly Th17 cells involved in RA pathogenesis are very susceptible to NK cell mediated cytotoxicity [2]. Thus when NK cell cytotoxicity was increased by blocking the inhibitory NK cell receptor CD94-NKG2A via anti-bodies, it resulted a decrease of Th17 numbers and in an arrest of RA disease progression [2,79]. A role for the inhibitory receptor CD94-NKG2A in RA was also suggested in other studies [79]. Not only does this receptor control NK cytotoxicity, it is also a known regulator of cytokine production by NK cells. In another study the blockage of CD94-NKG2A by antibodies lead to the increased production of IFN-γ by NK cells, resulting in an arrest of disease development [79]. TNF-α is a major contributor to RA pathogenesis and TNF- α blockers are already used as therapeutics against RA to reduce the chronic inflammation [80,81]. Hypothesised was that, IFN-γ can dramatically decrease the synthesis TNF-α in the synovial fluid causing an arrest of RA disease. However when the CD94-NKG2A inhibitory receptor was blocked, not only was there more production of IFN-γ, there was also an increase in production of TNF- α [79]. This increase in TNF- α production should theoretically lead to an increase of disease pathogenesis of RA, but TNF- α can also inhibit its own production to a certain extent [79]. Also IFN-γ has been shown to inhibit the differentiation of naïve T helper cells into Th17 cells [75]. Since blocking of CD94-NKG2A resulted in an arrest of disease development it may be possibly that the increased IFN-γ production has such an increased protective effect that it overshadows the destructive effects of more TNF- α production. Together

these data suggest that NK cells when activated have a protective role in RA pathogenesis. When NK cells are completely depleted in the collagen induced mouse model of RA this leads to an enhancement of disease, again suggesting a protective role for NK cells [78,82].

NK cells in RA do not only have a protective function, there are also reports which show that NK cells may enhance RA disease progression. NK cells have been shown to be involved in osteoclastogenesis and bone destruction in RA. Osteoclastogenesis is the formation of osteoclasts from monocytic precursor cells. These cells are mainly responsible for the destruction of bone seen in RA. In the collagen induced arthritis mouse model, NK cell depletion was associated with prevention of bone erosion and destruction [7]. The destruction of bone in RA is mediated through the action of two factors, RANKL (receptor activator of NFκB ligand) and M-CSF (macrophage colony-stimulating factor), which are highly expressed in the inflamed joint [83]. These factors induce the differentiation of monocyte precursors to osteoclasts. Targeted therapies with against RANKL and M-CF reduced the osteoclastogenesis and bone destruction [84,85]. Depletion of NK cells in the collagen induced arthritis mouse model gave similar results, suggesting that NK cells are involved in bone destruction [86]. It was found that NK cells express RANKL and M-CF and that NK cells *in vitro* can induce the differentiation of osteoclasts from monocyte precursors. Further, *in vivo* it was also seen that NK cells directly interact with CD14+ monocytes, the precursors of osteoclasts [86]. These findings show NK cells can enhance RA pathogenesis.

NK cells function in RA remains elusive, for both possible functions, protection against or enhancement of RA evidence has been found (as reviewed in[77]). However these results do not need to mutually exclude each other. First of all these studies were performed in the collagen induced arthritis mouse model. This model mimics the disease as it is seen in humans. Thus perhaps due to selection in the model, some functions of NK cells are not same in humans as in mice or due to selection some of the NK cells functions have become more prominent. NK cells could have an overall function in the enhancement of RA through their reduced cytotoxic activity. This reduced activity prevents the killing of Th17 cells, since target destruction of Th17 was only seen after blocking of the inhibitory receptor CD94-NKG2A. These functionally impaired NK cells in RA also induced bone destruction, suggesting that the overall function of NK cells is enhancing RA pathogenesis. Therefore NK cells most likely both protect against and enhance RA, but overall NK cells function to enhance RA pathogenesis.

Despite the contradictory reports of the role of NK cells in RA pathogenesis, NK cells can still be a very valuable target for therapeutics. Regardless the role of NK cells, manipulation of NK cells resulted in a change in disease development [2,86]. However depending on the focus of the research, cytotoxicity and Th17 cells or bone destruction, NK cells either enhanced or protected against RA. Regardless of the nature of NK cell function, possible therapies will all make use of NK receptors to manipulate NK cell function. In case for NK cells as protectors, therapy can include artificial stimulation of NK cells by blocking the inhibitory receptor CD/NKG2A. For NK cells enhancing RA, the expression of RANKL and M-CSF can be down regulated or blocked. If NK cells can both enhance and protect against RA at the same time a combination therapy might be effective, by inhibiting CD94-NKG2A, RANKL and M-CSF simultaneously. It is clear that NK cells contribute to RA and that they can be potential therapeutical target. However more insight into NK cell function in human RA is needed to determine the nature of the NK cell contribution to RA.

Discussion

To determine if NK cells can be potential drug targets for autoimmune disorders. Two models of autoimmune disorders were discussed to determine the role of NK cells in the development and pathology of autoimmune disorders. The precise mechanism explaining how NK cell function contributes to autoimmune disorders is currently unclear.

In the T1D model of autoimmunity, NK cells are the first immune cells causing diabetes through their killing of pancreatic β-cells, and blockage of the activating receptor NKp46 prevents T1D development. However activation of NK cells via CFA, increased cytokine production of NK cells which decreased the numbers of cytotoxic T cells and prevented T1D development [1]. Despite these contradictory results on NK cell function in T1D, NK cells have a mainly destructive function in T1D. Their protective function is only seen when the NK cells are stimulated by CIA mouse models, also NK cell function in T1D is impaired when compared to controls. Indicating that normally in T1D the impaired NK cell function actually contributes to disease pathogenesis. In RA mouse models, NK cells can, when activated via blockage of the CD94-NKG2A receptor, lyse Th17 cells and arrest disease progression [2]. Via the expression of RANKL and M-CSF on the cell surface of NK cells, osteoclastogenesis is induced which leads to bone destruction in RA [86]. As with T1D, NK cell functions in RA show contradictory functions, destructive and protective, however also here NK cells have a destructive function. Since only manipulation of NK cells resulted in the lysis of Th17 cells, which means that in RA the NK cells fail to attack these Th17 cells and thus contribute to disease pathogenesis. The overall function of NK cells in autoimmune diseases is thus disease enhancing. This view is supported by the fact that in many autoimmune diseases NK cells functions are impaired [12-15]. Also protective functions of NK cells were only seen after artificial activation or stimulation of NK cells, in TID after CIA treatment and in RA via the blockage of the CD94-NKG2A receptor.

NK cells function in autoimmunity and manipulation of their functions through inhibiting or activating their receptors results in arrest of disease development. Thus NK cells can be potential drug targets for autoimmune therapies. On possible therapy could be immunization against a specific natural killer cell receptor. Preventive immunization against natural killer cell receptors or ligands expressed on the NK cell surface can have negative effects on other immune functions of NK cells, since these receptors and ligands most likely also have other functions next to the known auto-immune function. When you block all of its functions it could possibly lead to disastrous effects, namely in activity against viruses or tumour surveillance and might even induce autoimmunity. These possible negative effects might be negligible for risk groups, like individuals with a genetic predisposition for autoimmunity development. Furthermore immunization would not benefit individuals already suffering from an autoimmune disease, because it takes quite some time before an effective immune response by the patient is achieved. In an autoimmune disease like T1D, the earlier the destruction of pancreatic β-cells can be halted the better the outcome for the patient is. Next to immunization, antibody treatment against NKRs and ligands expressed on the NK cell surface could be possible. The advantage of such treatment is that this can be done pre-emptive and also during disease development. Furthermore NK cell function is only temporarily manipulated, at least until the threat of autoimmune development or disease pathogenesis is gone. Which antibodies to use for therapies is dependent on the autoimmune disease, for T1D antibodies against the NKp46 receptor can be an effective therapy while for RA antibodies against RANKL and M-CSF would be beneficiary. Although that NK cells have a predominantly destructive role in autoimmunity, and therapies most focus on inhibition of NK cell function, the activation of NK cells via antibodies or an immune stimulator also resulted in positive effects on disease development. In RA immune stimulation of NK cells increased the cytotoxicity of the NK cells and in the lysis of Th17 cells. Perhaps combinatory immunotherapies can also be effective. Inhibition or blockage of the specific receptor or ligand expressed at the NK cell surface will lead to a reduction of the enhancement of autoimmune disease by NK cells. At the same time NK cells are stimulated through inhibition of an inhibitory receptor or via an immunostimulus cause for the protective roll of NK cells in autoimmunity to be activated. However it is not very clear if such therapies

would also work in patients with established autoimmunity. NK cells do still function in the autoimmunity, but most of the destructive damage is now mediated by cells of the adaptive immune system, auto-reactive T and B cells. Perhaps other combinatory immunotherapies are needed, first manipulate auto-reactive T or B cell function, and then also NK cell function Combination therapy where both the innate and the adaptive immune system is manipulated might help in patients with established autoimmunity. However all of these postulated therapies are highly speculative, since most have yet to be tested on human autoimmune disorders, but it is clear that NK cells can be used as potential drug targets in autoimmune disorders.

NK cell function in autoimmune diseases is predominantly enhances disease and manipulation NK cell function leads to prevention and arrest of autoimmune disease. NK cells thus can be good drug targets in autoimmunity disorders. However autoimmunity is a multifactorial disease caused by a combination of factors, like inflection, genetic predisposition, cytokines, NK cells and stress, that together cause for autoimmune disorders to develop. Therefore focussing therapies only on one cell type will not be as successful as combinatory therapies. It remains important to see NK cell function only as one factor in large complex of contributing factors to disease development. A better understanding of all these contributing factors will lead to a collective understanding of autoimmune diseases.

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