Review Article
Natural Killer Cells in the Orchestration of Chronic Inflammatory Diseases

Luca Parisi,1 Barbara Bassani,2 Marco Tremolati,1 Elisabetta Gini,3 Giampietro Farronato,1 and Antonino Bruno2

1Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy
2Scientific and Technological Pole, IRCCS MultiMedica, Milan, Italy
3Department of Biotechnology and Life Sciences (DBSV), University of Insubria, Varese, Italy

Correspondence should be addressed to Luca Parisi; luca.parisi@unimi.it

Received 4 October 2016; Revised 4 January 2017; Accepted 18 January 2017; Published 27 March 2017

Academic Editor: Margarete D. Bagatini

Copyright © 2017 Luca Parisi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Inflammation, altered immune cell phenotype, and functions are key features shared by diverse chronic diseases, including cardiovascular, neurodegenerative diseases, diabetes, metabolic syndrome, and cancer. Natural killer cells are innate lymphoid cells primarily involved in the immune system response to non-self-components but their plasticity is largely influenced by the pathological microenvironment. Altered NK phenotype and function have been reported in several pathological conditions, basically related to impaired or enhanced toxicity. Here we reviewed and discussed the role of NKs in selected, different, and “distant” chronic diseases, cancer, diabetes, periodontitis, and atherosclerosis, placing NK cells as crucial orchestrator of these pathologic conditions.

1. Introduction

Inflammation is now considered a crucial hallmark of chronic disorders, including cardiovascular [1], neurodegenerative diseases [2–4], diabetes [5, 6], metabolic syndrome [7, 8], and cancer [9–11]. Inflammation acts as a relevant orchestrator in their insurgence, development, and progression [1, 2, 6, 10]. Inflammatory cells, which comprise cells from innate ad adaptive immunity, are characterized by different phenotype and functions, which involve direct (by contact) or distant (by soluble factors) interaction with their target cells [12–14]. Immune cells, form either innate or adaptive immunity, given their cellular plasticity, have been reported to acquire an altered phenotype upon different stimuli. This has been described for diverse immune cell type, including macrophages (M), neutrophils (N), myeloid-derive-suppressor cells (MDSC), dendritic cells (DC), natural killer (NK) cells, and T cells [12–14]. Immune cell altered functions include attenuation of targeting/killing activities, tolerogenic/immunosuppressive behaviour, and the acquisition of proangiogenic functions. These alterations, occurring at both tissue levels and systemically, are finely tuned by the (chronic) pathological environment. Here, we focused on NKs as key orchestrator in inflammatory chronic diseases, such cancer, type 1 diabetes, periodontitis, and atherosclerosis. The aim was to discuss how such very different pathologies, with diverse aetiology and photogenic mechanisms, share a common and relevant hallmark, such inflammation, dissecting whether NK cells act as crucial orchestrators in the induction and progression of the conditions selected.

NKs are innate lymphoid cells, primarily involved in the host defence against infection and in the process of tumour immunosurveillance. A part from their crucial role in those processes, NK cells are involved in the graft-versus-host disease, the regulation of haematopoiesis, and exert regulatory effects on the adaptive immune cell counterpart [15]. Virus infected and tumour transforming cells share the feature of low/null expression of the MHC-I molecule, representing one of the mechanisms through which NK cells are able to recognize target/non-self-cells. Nevertheless, this mechanism alone does not trigger cytotoxicity, unless it is combined with the altered expression of other molecules
on the target cell surface, acting as activatory ligands. NK cells are equipped with surface receptors that trigger cell activation (immunoreceptor tyrosine-based activation motifs (ITAM)) or inhibition (immune tyrosine-based inhibitory motifs (ITIM)) [16]. NK cells also directly contribute to adaptive immune responses, interacting with DCs and by triggering T cell responses. Induction of DC maturation to produce TNF-α and IL-12 and upregulation of costimulatory ligands are triggered by NK cells [17]. Moreover, NK cells proliferate and acquire cytotoxic activity and the capacity to produce IFN-γ through the interaction with DCs [18]. Apart from activation of other cells of innate immunity, NK cells also enhance induction of CD8+ T cell responses that is influenced by NK-released IFN-γ, which promotes antigen processing and presentation to T cells and T helper type 1 (Th1) cell polarization [19]. Two major circulating human NK cell subsets have been characterized based on the expression of surface antigens: CD56, an isoform of the human neural cell adhesion molecule, and CD16, the low-affinity Fc receptor necessary for the antibody-dependent cellular cytotoxicity (ADCC). Peripheral NK cells are predominantly CD56dimCD16+ cytotoxic NK cells acting mainly through the release of perforin, a membrane pore-forming toxin, and granzyme which activates the apoptotic cascade on target cells. However, approximately 5% of circulating NK cells show a CD56brightCD16− phenotype. These cells can produce high levels of some cytokines. Upon activation, CD56brightCD16− NK cells release IFN-γ and TNF-α, and they kill target cells more efficiently [20]. Within the developing decidua, a third NK subset is found, the decidual NK cell (dNK). dNK cells display a CD56superbrightCD16− phenotype [21] and are closely linked with vascularization of the decidua in both humans and mice. dNK cells physiologically produce VEGF, PlGF, and IL-8, are poorly cytotoxic, and are associated with the induction of CD4+ T regulatory (Treg) cells [22, 23] (Figure 1).

2. Natural Killer Cells and Cancer

Strong evidences suggest that the presence of inflammatory cells within the tumour microenvironment (TME) plays a crucial role in the development and/or progression of human cancers [10, 12, 24–27]. Among the host-dependent biological features of the tumour hallmarks, defined by Hanahan and Weinberg [28], there are “evading immune destruction” and “tumour-promoting inflammation,” which together with the immune orchestration of angiogenesis point out the key role of the immune system in neoplastic diseases [9, 10, 12, 24].

Alterations in NK cell activity have been described in different type of cancer and are associated with the induction of a tolerogenic and less/poor cytotoxic functions with decreased expression of the activatory receptor NKG2D, altered degranulation, and release of perforin and granzyme. Recently, Bruno et al. identified a new NK cell subset in tissue and peripheral blood of non-small-cell lung cancer (NSCLC) patients, termed, respectively, tumour infiltrating (TINKs) and tumour associated (TANKs) NKs, which are able to promote angiogenesis [29, 30]. NSCLC TINK/TANKs are characterized by a decidual-like phenotype CD56brightCD16− VEGFhighPlGFhighIL-8−IFN-γlow, able to promote endothelial cell migration and induction of capillary-like structures [29, 30].

Several TME released components, including TGF-β, hypoxia, and adenosine, mostly shared with the decidual tissues, are implicated in NK cell response against tumours [31] (Figure 2).

TGF-β is one of the numerous TME factors involved in the induction of immune cell polarization [32] and is expressed at high levels both in the tumour microenvironment and in the decidua [29]. During carcinogenesis, TGF-β acts as a tumour suppressor, by inhibiting tumour cell replication and favouring apoptosis [33, 34], while at later stages of tumour progression it exerts protumourigenic...
Figure 2: Soluble factors within the tumour microenvironment shaping NK cell functions. Several molecules and soluble factors within the tumour microenvironment, including TGF-β, hypoxia, adenosine, acidic of the environment, and tumour exosomes (TEXOs), can inhibit NK response against tumours either by interfering with NK cell direct/cytokine mediated tumour cell lysis or by supporting tumour angiogenesis.

effects that include tumour survival, induction of epithelial-mesenchymal transition (EMT), enhanced tumour invasion, and immunosuppressive and proangiogenic activities [32–34]. TGF-β has been found to polarize the CD56dim CD16+ peripheral NK cells towards a decidual like phenotype, defined as CD56bright CD16− and KIR+ CD9+ CD49a+ [29,35–37]. TGF-β has been shown to inhibit CD16 mediated human NK cell IFN-γ production and ADCC though SMAD3 [36]. Bruno et al. demonstrated that TGF-β significantly contributes in the induction of the angiogenic-switch of NK cells from healthy individuals [30], promoting the induction of the TINK/TANK CD56bright CD16− VEGFhigh PlGFhigh IL-8* INFγ low phenotype in vitro.

A hypoxic microenvironment is another common feature shared between the decidua and the TME [38, 39]. A combination of TGF-β hypoxia and 5-aza-2'-deoxycytidine, a demethylating agent, has been found to convert FACS sorted peripheral blood CD56dim CD16+ NK cells into dNKs, characterized by low cytotoxicity and high expression levels of VEGF, the CD9 dNK marker, and KIRs [36].
Adenosine is a soluble immunomodulatory molecule acting through adenosine receptors expressed on diverse immune cell types, including NK cells [40, 41]. Up to 20-fold increases in the adenosine content in extracellular fluid of solid carcinomas have been reported [42]. Adenosine accumulation is partially associated with hypoxia and its release in the extracellular environment and can impair NK cell cytolytic activities by decreasing TNF-α secretion (following IL-2 stimulation), decreasing cytotoxic granule exocytosis, and attenuating perforin and Fas ligand-mediated cytotoxic activity as far as cytokine release. Most of these effects are attributed to stimulation of the cyclic adenosine monophosphate/protein kinase A (PKA) pathway, following the binding of adenosine to A2A receptors on NK cells [43].

Recently, great interests arise on tumour released vesicles, including exosomes, in shaping immune cell response [44, 45]. Exosomes are small (40 to 110 nm) membrane vesicles of endocytic origin which are actively secreted from several cell types. Exosome content includes a variety of biologically active molecules such as proteins, mRNAs, and miRNAs reflecting the cell of origin. They probably mediate a range of local and systematic functions, including immune stimulation or suppression, cell-to-cell communication, delivery of proteins, and genetic material, including miRNA, tumour immune escape, and tumour cell communication [46, 47]. Tumour derived exosomes appear to regulate NK cells impairing their killing activity by downregulating perforin/granzyme production and/or NKG2D ligand expression [48, 49]. Exosome release could explain the effects of tumours on the polarization of peripheral NK cells towards TANK phenotype. The NKG2D/NKG2DL system plays an important role in tumour immune surveillance [42, 48, 49]. There are convincing evidences that exosomes derived from diverse cancer cell lines, including mesothelioma, breast, and prostate cancer cells, express NKG2D ligands, and thereby downregulate NKG2D expression on NK cells and CD8+ T cells, resulting in impaired cytotoxic effector functions [48–50]. It has also been shown that leukaemia/lymphoma T and B cells secrete NKG2D ligand-expressing exosomes with the ability to impair the cytotoxic potency of NK and T cells from healthy donors [44, 45].

Recently, STAT5 has been proposed as a key regulator in NK cells and demonstrated that STAT5 acts as a molecular switch from tumour surveillance to tumour promotion [39]. Consistent with its function as the major STAT protein downstream of IL-7, IL-2, and IL-15, Gotthardt et al. reported STAT5 role in tumour angiogenesis showing that Stats−/− Ncr1−/−CreTg8.Vav-Bcl2−/− mice displayed an increased tumour growth compared with wild-type mice [51]. In addition, production of VEGF by NK cells is higher in STAT5−/− mice compared with wt-mice. To elucidate the role of VEGF production in NKp46+ cells, Gotthardt and colleagues established VegfA−/−Ncr1−/−CreTg8 mice, characterized by NKp46− VEGF− cells. In v-abl− tumour, RMA-S, and A-MuLV-induced leukaemia tumour models, they showed a significant reduction of tumour burden and fewer CD31+ blood vessels in tumours.

3. Natural Killer Cells in Type 1 Diabetes

Type 1 diabetes (T1D), an autoimmune disease characterized by almost complete beta cell destruction and hyperglycaemia, accounts for only about 5–10% of all cases of diabetes, whereas its incidence is dramatically increasing worldwide over the last 50 years [52]. Different immune cells, such macrophages and dendritic and T cells, have been suggested to play crucial roles in type 1 diabetes pathogenesis [53–55]. The contribution of autoreactive T cells to the destruction of pancreatic β cells as a consequence of an immunologically mediated destruction of the pancreatic tissues has been proposed as the key pathogenic mechanisms in type 1 diabetes [56, 57]. Nevertheless, diverse inflammatory cells, from both innate and adaptive immunity, interact with the pancreatic parenchyma, supporting the overall inflammatory state in T1D. NKs cells represent the major source of IFN-γ, a Th1 proinflammatory cytokine acting as a master regulator of different immune cell response. High release of IFN-γ within the pancreatic tissues in T1D patients may significantly contribute to the excessive, uncontrolled, and unresolved autoimmune response mediated by autoreactive T cells. While NK cell response against autologous pancreatic islet has been reported in vitro [58], contrasting results have been reported in in vivo models.

Two in vivo studies correlate NK cells to diabetes progression. In the first study (Figure 3(a)), an in vivo model of coxsackievirus B4- (CVB4-) induced diabetes was employed, showing that NK antiviral defence, raised by beta cells in response to IFNs, resulted in a reduced permissiveness to infection and subsequent natural killer (NK) cell-dependent death [59]. Another in vivo study (Figure 3(b)), using a T cell receptor transgenic model where T1D was induced via anti-CTLA-4 mAb treatment, revealed that higher frequency of NK cells exited in aggressive insulitis, resulting in b-islet cell destruction [60].

Conversely, there are several reports supporting a protective role exerted by NK cells, in NOD mice undergoing complete Freund’s adjuvant (CFA). In this work, NOD/SCID mice immunized with CFA recover its protective effects when CD3−DX5+ NKs were adaptively transferred into animal recipients, by downregulating autoreactive T cell response [61] (Figure 3(c)).

Whether the murine model employed is relevant for the NK cell behaviour detected in the context of T1D is still debated. For example, NOD mice are characterized by an unusual genetic composition in the genomic regions that influence NK cell activity.

The NKG2D activatory receptor has been demonstrated to be overexpressed in NOD NKs due to the overexpression of its Rae-1 ligand. Further, diverse NK cell inhibitory receptors have been found to be differentially expressed in NOD mice as compared to C57BL/6 control animals [62]. Altogether, these genetic peculiarities may explain the low NKs activity detected in NOD mice [62, 63].

Moving to humans (Figure 3(d)), contrasting results have been reported as well (Figure 2). Most of them documented low number of circulating NK cells in T1D patients [64–66] or a functional altered state [67, 68]. The major concern
Figure 3: Natural killer cells in type 1 diabetes. Several in vivo studies correlate NK cells to diabetes progression: an in vivo model of coxsackievirus B4 (CVB4-) induced diabetes showed that NK antiviral defence resulted in a reduced permissiveness to infection and subsequent NK cell-dependent death (a). Anti-CTLA-4 mAb treatment of T cell receptor transgenic mice demonstrated that higher frequency of NK cells induces aggressive insulitis, resulting in β-islet cell destruction (b). A protective role of NK cells was reported in NOD mice undergoing complete Freund’s adjuvant (CFA). NOD/SCID mice immunized with CFA recover its protective effects when CD3^- DX5^+ NKs are adoptively transferred into animal recipients, by downregulating autoreactive T cell response (c). In human samples, lower expression of the NCR NKp30 and NKp40 was detected in type 1 diabetic patients as compared with control. Type 1 diabetic patients display an increased frequency of KIR gene haplotypes, including the activating KIR2DS3 gene, with a genetic interaction between the KIR and HLA complexes (d).

Regarding these studies is that, even if NK cells were directly isolated from T1D patients, the detection of functional alteration was performed by assessing NK cell cytolysis on K562 tumour cells.

Rodacki et al. investigated the frequency and activatory state of peripheral blood NK cells in individuals with T1D at different stages (recent versus long-standing onset) [69, 70]. No significant difference between the activatory state, as detected by IFN-γ and perforin release, was observed between NK cells derived from either recent or long-standing T1D patients. In contrast, lower expression of the NCR NKp30 and NKp40 was detected in NK cell isolated from long-standing type 1 as compared with control subjects. Further, gene expression analysis revealed that type 1 diabetic patients display an increased frequency of KIR gene haplotypes, including the activating KIR2DS3 gene, with a genetic interaction between the KIR and HLA complexes [69, 70].

4. Natural Killer Cells in Periodontitis

Periodontitis, defined as the inflammation of the periodontium involving the supporting tissues of the teeth, affects as much as 80% of the middle-aged population; by comparison, the prevalence of aggressive periodontitis reaches up to 1–1.5% [71].

The role of NK cells in periodontitis has been poorly investigated; however decreased Th cells and upregulation of NK cells during CP have been documented [72].

The role of NKs in periodontitis represents a still debated issue. Indeed, contrasting results have been reported by using human specimens. While some of them showed a relationship between NK number, phenotype, and periodontal state [73–76], others reported no significant correlation [73, 77].

Relevant increase of CD57 NK cells has been observed by Fujita et al. and related to periodontal diseases progression as a consequence of an unresolved immune response within periodontal tissues [73–76].
Contrast studies conducted by Fujita et al. and Cobb et al. revealed relatively low numbers of natural killer cells in chronic gingivitis and periodontitis samples, as compared to healthy subjects’ correlation [73, 77]. Conversely, in vitro studies have focused on the interaction between NK cells and the main bacterial species involved in the pathogenesis of periodontitis (Figure 4), like A. actinomycetemcomitans, P. gingivalis, and F. nucleatum [78].

Direct recognition of Fusobacterium nucleatum, a gram-negative anaerobe microorganism ubiquitous to the oral cavity [79], by the NK cell natural cytotoxicity receptor NKp46, has been reported to aggravate periodontal disease [78] (Figure 4(A)).

Actinobacillus actinomycetemcomitans, a gram-negative bacterium which has been associated with severe oral infections [80], has been shown to elicit rapid gamma interferon responses by natural killer cells, via dendritic cell stimulation [81] (Figure 4(B)). Increased type 1 cytokine production by both dendritic cells and NK cells, following exposition to P. gingivalis, has been described, resulting in increased P. gingivalis-specific IgG2 [81] (Figure 4(C)). Aggregatibacter actinomycetemcomitans, a gram-negative anaerobic bacterium strongly associated with localized aggressive periodontitis [82], has been reported to indirectly induce CD2-like receptor activating cytotoxic cells (CRACC) on NK cells, via activation of dendritic cells and subsequent IL-12 signalling [83]. CRACC induction was reported to be more significantly pronounced in aggressive than chronic periodontitis and positively correlated with periodontal disease severity, subgingival levels of specific periodontal pathogens, and NK cell activation in vivo [83] (Figure 4(D)).

Other relevant mechanisms driving NK cell contribution to periodontitis involve ncr1 receptor recognition of still unknown ligands on F. nucleatum surface. This interaction resulted in TNF-α secretion that on one hand leads to tissue damage by stimulating prostaglandin E2 release from monocytes and fibroblasts, secretion of metalloproteinases that degrade extracellular matrix (ECM) proteins, and on the other hand induces osteoclast differentiation and activation by increasing RANKL expression and the suppression of osteoprotegerin, a cytokine receptor that belongs to tumour necrosis factor (TNF) receptor superfamily expression in osteoblasts, resulting in alveolar bone resorption (E).
those related to bacterial infections, T2DM [67], smoking habits [84] have been included as related conditions.

Fanconi anemia (FA), an autosomal recessive disorder characterized by progressive pancytopenia and congenital malformation of the skeleton [85], periodontitis, and ginglyvi-
tis, represents common inflammatory states in patients with
FA [85]. Natural killer (NK) cell numbers and function have
been reported to be decreased in some FA patients and this
was associated with impairment in the differentiation process
of the NK cells subsets [85, 86]. Myers et al. showed perforin
and granzyme reduced content in NK cells from children with
FA as compared to controls [87].

Zeidel et al. reported that smokers without chronic
obstructive pulmonary disease (COPD) showed impaired
NK cytotoxic activity in peripheral blood and alteration in
systemic production of pro- and anti-inflammatory cytokines
[84].

A study performed on Papillon-Lefèvre syndrome (PLS),
an autosomal recessive disorder that exited in aggressive
periodontitis [88], revealed NK cell anergy, as compared to
healthy subjects with impairment of NK cell cytotoxic
function [89].

5. Natural Killer Cells in Atherosclerosis

Atherosclerosis is a chronic inflammatory disease affecting
elastic and large muscular arteries that are characterized by
lesions containing cholesterol, immune cells, smooth muscle
cells, and necrotic cores. Macrophages, dendritic cells, and
T cells represent the major immune cells populations within
developing lesions, even if other immune cell components
are involved, including NK cells [90]. Indeed, NKs have been
observed within atherosclerotic plaques in humans as far as in
mice [91, 92] and it has been demonstrated that in advanced
atherosclerotic lesions they mostly localized in the necrotic
core adjacent tissues, deep within plaques, and in shoulder
regions [90].

It has been suggested that several cytokines and chemok-
ines within lesions may be directly involved in NK cell
recruitment towards atherosclerotic plaque. Among all,
monocyte chemoattractant protein-1 (MCP-1) [93] as well as
fractalkine (CX3CL1) has been shown as relevant cytokines
able to enhance NK cell migration and activation resulting
in an increased IFN-γ release [94]. In addition, IL-
15, IL-12, IL-18, and IFN-α, which represent major NK cell
chemoattractants, have been shown to promoteatherogenic
process, potentially activating NK cells, or promoting their
crosstalk with other immune cells, including DC and mono-
cytes/macrophages [95–97] (Figure 5(a)). NKs have been
shown to participate in atherosclerosis via activatory recep-
tors that recognize MHC-I molecules (MICA and MICB) [98]
and by releasing IFN-γ, a proinflammatory cytokine [99].
In this context, it was demonstrated that oxidized low-density
lipoprotein (LDL) receptor null (ldlr−/−) mouse model also character-
ized by the impairment of NK cell functionality through the
expression of a transgene encoding for Ly49A. They
demonstrated that, even if no difference in either serum
total cholesterol concentrations or lipoprotein cholesterol
distribution was observed between the two groups of mice,
in Ly49A transgenic group the deficiency of functional NK
cells significantly reduced the size of atherosclerosis by 70%
in cross-sectional analysis of the aortic root and by 38% in the
intimal surface of the aortic arch [92, 99] (Figure 5(b)).

Selathurai et al. demonstrated that treatments with anti-
Asialo-GM1 in ApoE(−/−) mice resulted in NK cells depletion
without affecting other lymphocytes ratios, associated with
reduced atherosclerosis (Figure 5(c)). These effects have been
shown to be independent from plasma lipids. Moreover
NKs isolated from mouse spleens for adoptive transfer into
lymphocyte-deficient ApoE(−/−)Rag2(−/−) IL2rg(−/−) mice
confirmed the proatherogenic activity of NK cells. Further,
the transfer of IFN-γ-deficient NK cells, but not granzyme
B and perforin-deficient NK cells, resulted in an increased
lesion size in the lymphocyte-deficient ApoE(−/−) mice as in
wild-type NK cells. Necrotic core was increased by wild-type
NK cells, whereas no changes were observed with perforin-
and granzyme B-deficient NK cell transfer [98] (Figure 5(d)).

Cheng et al. showed that combined B, T, and NK
cell deficiency accelerates atherosclerosis in BALB/c mice,
demonstrating the impact of lymphocytes, including NKs, on
lipoprotein metabolism along with the relevant contribution
of lymphocyte subsets in plaque composition in atheroscle-
rosis [101].

Recent studies have suggested that not only might the
presence of NKs be considered in atherosclerosis progres-
sion, but also more importantly their ability to influence
other immune cells should be evaluated. Several evidences
demonstrated that NKs within atherosclerotic plaque are
activated by dendritic cells. NK-released cytokines are able in
turn to promote DC maturation, leading to an exacerbation
inflammatory response. NK/DC crosstalk might be envisaged
as a potential interaction occurring within atherosclerotic
lesions, which might worsen disease progression [102] (Fig-
ure 5(a)). In fact, the crosstalk between activated dendritic
cells/macrophages and NK cells induces IFN-γ release by
NK cells that in turn promotes metalloproteinases (MMPs)
secretion from cDCs and Mφ. Activated Mφ produce TNF-
α increasing enhance endothelial cell adhesion molecules
and MMPs can damage the extracellular matrix leading to
atherosclerotic plaque destabilization [102] (Figure 5(a)).

Indirect evidence that NK cells might contribute to
atherosclerotic disease is also provided by clinical obser-
vations in atherosclerotic patients. Recently, in a cohort of
124 patients it has been demonstrated that increased NK
numbers were observed in the arm of the study including
those patients with complications, suggesting NK cell direct
contribution in atherosclerosis progression [103]. Similarly,
in elderly atherosclerotic patients, an increased number of total
circulating NKs characterized by an impaired cytotoxicity
were shown [104]. According to these evidences, a significant
Figure 5: Proatherosclerosis role of natural killer cells. NK ability to induce atherosclerosis has been reported in several murine models and in humans. Several cytokines and chemokines within atherosclerotic lesions are supposed to promote NK recruitment towards atherosclerotic plaque, including monocyte chemotactic protein-1 (MCP-1), fractalkine (CX3CL1), IL-15, IL-12, IL-18, and IFN-α that on one hand enhance NK cell migration and on the other hand induce NK activation resulting in an increased IFN-γ release. Moreover, these cytokines as far as oxidized LDL also promote the NK crosstalk with other immune cells, that is, dendritic cells and macrophages. DCs activated NKS by releasing IL-12 that in turn induce the production of IFN-γ by NKS that are able to lyse smooth muscle cells. In addition, IFN-γ released NK cells that in turn promote metalloproteinases (MMPs) secretion from cDCs and MΦ (a). In a chimeric atherosclerosis-susceptible low-density lipoprotein (LDL) receptor null (ldl-/-/−) mouse model, characterized by the impairment of NK cell functionality through the expression of a transgene encoding for Ly49A, it has been demonstrated that even if no difference in either serum total cholesterol concentrations or lipoprotein cholesterol distribution was observed between the two groups of mice, in Ly49A transgenic group, the deficiency of functional NK cells significantly reduced the size of atherosclerosis by 70% in cross-sectional analysis of the aortic root and by 38% in the intimal surface of the aortic arch (b). The administration of anti-Asialo-GM1 antibodies in ApoE(−/−) mice induces a NK cells depletion leading to an attenuation of atherosclerosis (c). The transfer of NK cells isolated from murine spleen into ApoE(−/−)Rag2(−/−)IL2rg(−/−) mice induces an enhancement of atherosclerosis (d).
reduction of CD56<sup>dim</sup> NK cells and a concomitant loss of NK cell function in terms of cytolytic activity were also found in patients with unstable coronary artery disease (CAD) [105].

6. Conclusion

The contribution of natural killer cells to inflammatory disease insurgence and progression represent an intriguing topic for both basic scientists and clinicians. It is now clear that immune cell plasticity within the pathological microenvironment acts not only at local tissue but also at systemic levels. Several studies performed in different and distant pathologies like cancer, diabetes, and dental disorders that share an inflammatory state as a crucial hallmark showed altered NK cell activity at both local and systemic levels. This will be crucial to propose NK cells as potential circulating biomarkers to early detect diverse syndrome and/or predict further outcome.

Some studies, here discussed, supported the evidence that altered NK cell activities (enhanced/uncontrolled cytolysis versus impaired cytolytic functions) are associated with protective or deleterious effects. This knowledge suggests that further studies, requiring proper animal models and translation into human, are necessary to clarify the contribution of NK cells to the progression of inflammatory-related pathologies, aiming at identifying potential modulators able to shape NK cells, according to the pathological context. Finally, considering their direct and indirect (crosstalk with other arms on innate and adaptive immunity) contribution to inflammatory conditions, NK cells can be placed as relevant orchestrators in chronic diseases.

**Abbreviations**

ADCC: Antibody-dependent cellular cytotoxicity  
A-MuLV: Abelson murine leukaemia virus  
CD: Cluster of differentiation  
CFA: Complete Freund's adjuvant  
COPD: Chronic obstructive pulmonary disease  
CP: Chronic periodontitis  
CRACC: CD2-like receptor activating cytotoxic cells  
CTLA: Cytotoxic T-lymphocyte antigen  
CVB4: Coxsackievirus B4  
CX3CL1: Fractalkine  
DC: Dendritic cells  
dNK: Decidual NK cell  
ECM: Extracellular matrix  
FA: Fanconi anemia  
FACS: Fluorescence-activated cell sorting  
HLA: Human leukocyte antigen  
IFN: Interferon  
IL: Interleukin  
ITAM: Immunoreceptor tyrosine-based activation motifs  
ITIM: Immune tyrosine-based inhibitory motifs  
KIR: Killer cell immunoglobulin-like receptor  
LDL: Low-density lipoprotein  
Ly49A: Killer cell lectin-like receptor subfamily A  
M: Macrophages  
MΦ: Naïve macrophages  
MCP-1: Monocyte chemoattractant protein-1  
MDSC: Myeloid-derived-suppressor cells  
MHC: Major histocompatibility complex  
MICA: MHC class I polypeptide-related sequence A  
MICB: MHC class I polypeptide-related sequence B  
MMPs: Metalloproteinases  
mRNA: Messenger RNA  
N: Neutrophils  
NCR: Natural cytotoxicity receptors  
NK: Natural killer  
NKG: Natural killer group  
NOD: Nonobese diabetic  
NOD/SCID: Nonobese diabetic/severe combined immunodeficiency  
NSCLC: Non-small-cell lung cancer  
PKA: Protein kinase A  
P1GF: Placental growth factor  
PLS: Papillon-Lefèvre syndrome  
Rae: Retinoic acid early inducible  
RANKL: Receptor activator of nuclear factor-κB ligand  
RMA-S: Rejected an MHC class I-deficient tumour cell line  
SMAD: Small mother against decapentaplegic  
STAT: Signal transducers and activators of transcription  
Th: T helper  
TANKs: Tumour associated natural killer cells  
TINKs: Tumour infiltrating natural killer cells  
TGF: Transforming growth factor  
TME: Tumour microenvironment  
TNF: Tumour necrosis factor  
Treg: T regulatory  
TID: Type 1 diabetes  
T2DM: Type 2 diabetes mellitus  
v-abl: Abelson murine leukaemia viral oncogene homolog 1  
VEGF: Vascular endothelial growth factor.

**Disclosure**

Luca Parisi and Barbara Bassani are co-first authors and Giampietro Farronato and Antonino Bruno are co-last authors.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

Luca Parisi, Barbara Bassani, Giampietro Farronato, and Antonino Bruno share equal contribution.
Acknowledgments

Barbara Bassani and Elisabetta Gini are students of the Ph.D. program in Biotechnology, Biosciences, and Surgical Technologies, School in Biological and Medical Sciences, University of Insubria. Antonino Bruno was a FIRC (Fondazione Italiana per la Ricerca sul Cancro) fellow and is currently a fellow for Fondazione Umberto Veronesi (FUV).

References

[1] P. Libby, “Inflammation and cardiovascular disease mechanisms,” The American Journal of Clinical Nutrition, vol. 83, no. 2, pp. 456S–460S, 2006.
[2] S. Amor, F. Puentes, D. Baker, and P. Van Der Valk, “Inflammation in neurodegenerative diseases,” Immunology, vol. 129, no. 2, pp. 154–169, 2010.
[3] C. K. Glass, K. Sajio, B. Winner, M. C. Marchetto, and F. H. Gage, “Mechanisms underlying inflammation in neurodegeneration,” Cell, vol. 140, no. 6, pp. 918–934, 2010.
[4] T. Wyss-Coray and L. Mucke, “Inflammation in neurodegenerative disease—a double-edged sword,” Neuron, vol. 35, no. 3, pp. 419–432, 2002.
[5] M. Y. Donath, “Targeting inflammation in the treatment of type 2 diabetes: time to start,” Nature Reviews Drug Discovery, vol. 13, no. 6, pp. 465–476, 2014.
[6] N. Esser, S. Legrand-Poels, J. Piette, A. J. Scheen, and N. Paquot, “Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes,” Diabetes Research and Clinical Practice, vol. 105, no. 2, pp. 141–150, 2014.
[7] K. Esposito and D. Giugliano, “The metabolic syndrome and inflammation: association or causation?” Nutrition, Metabolism and Cardiovascular Diseases, vol. 14, no. 5, pp. 228–232, 2004.
[8] R. Monteiro and I. Azevedo, “Chronic inflammation in obesity and the metabolic syndrome,” Mediators of Inflammation, vol. 2010, Article ID 289645, 10 pages, 2010.
[9] F. R. Balkwill and A. Mantovani, “Cancer-related inflammation: common themes and therapeutic opportunities,” Seminars in Cancer Biology, vol. 22, no. 1, pp. 33–40, 2012.
[10] L. M. Cossens and Z. Werb, “Inflammation and cancer,” Nature, vol. 420, no. 6917, pp. 860–867, 2002.
[11] C. I. Diakos, K. A. Charles, D. C. McMillan, and S. J. Clarke, “Cancer-related inflammation and treatment effectiveness,” The Lancet Oncology, vol. 15, no. 11, pp. e493–e503, 2014.
[12] A. Bruno, A. Pagani, L. Pulze et al., “Orchestration of angiogenesis by immune cells,” Frontiers in Oncology, vol. 4, article 131, 2014.
[13] A. Mantovani, M. A. Casatella, C. Costantini, and S. Jaillon, “Neutrophils in the activation and regulation of innate and adaptive immunity,” Nature Reviews Immunology, vol. 11, no. 8, pp. 519–531, 2011.
[14] A. Sica and A. Mantovani, “Macrophage plasticity and polarization: In vivo veritas,” Journal of Clinical Investigation, vol. 122, no. 3, pp. 787–795, 2012.
[15] B. R. Blazar, W. J. Murphy, and M. Abedi, “Advances in graft-versus-host disease biology and therapy,” Nature Reviews Immunology, vol. 12, no. 6, pp. 443–458, 2012.
[16] L. L. Lanier, “Turning on natural killer cells,” Journal of Experimental Medicine, vol. 191, no. 8, pp. 1259–1262, 2000.
[17] F. Gerosa, B. Baldani-Guerra, C. Nisii, V. Marchesini, G. Carra, and G. Trinchieri, “Reciprocal activating interaction between natural killer cells and dendritic cells,” Journal of Experimental Medicine, vol. 195, no. 3, pp. 327–333, 2002.
[18] N. C. Fernandez, A. Lozier, C. Flamet et al., “Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo,” Nature Medicine, vol. 5, no. 4, pp. 405–411, 1999.
[19] R. Mocikat, H. Braumüller, A. Gumy et al., “Natural killer cells activated by MHC class I-low targets prime dendritic cells to induce protective CD8 T cell responses,” Immunity, vol. 19, no. 4, pp. 561–569, 2003.
[20] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, “Functions of natural killer cells,” Nature Immunology, vol. 9, no. 5, pp. 503–510, 2008.
[21] P. Le Bouteiller, “Human decidual NK cells: unique and tightly regulated effector functions in healthy and pathogen-infected pregnancies,” Frontiers in Immunology, vol. 4, article 404, 2013.
[22] S. M. Blois, B. F. Klapp, and G. Barrientos, “Decidualization and angiogenesis in early pregnancy: unravelling the functions of DC and NK cells,” Journal of Reproductive Immunology, vol. 88, no. 2, pp. 86–92, 2011.
[23] J. Hanna, D. Goldman-Wohl, Y. Hamani et al., “Decidual NK cells regulate key developmental processes at the human fetomaternal interface,” Nature Medicine, vol. 12, no. 9, pp. 1065–1074, 2006.
[24] F. R. Balkwill, M. Capasso, and T. Hagemann, “The tumor microenvironment at a glance,” Journal of Cell Science, vol. 125, no. 23, pp. 5591–5596, 2012.
[25] A. Bruno, A. Pagani, E. Magnani et al., “Inflammatory angiogenesis and the tumor microenvironment as targets for cancer therapy and prevention,” Cancer Treatment and Research, vol. 159, pp. 401–426, 2014.
[26] S. M. Crusz and F. R. Balkwill, “Inflammation and cancer: advances and new agents,” Nature Reviews Clinical Oncology, vol. 12, no. 10, pp. 584–596, 2015.
[27] D. M. Noonan, A. De Lerma Barbaro, N. Vannini, L. Mortara, and A. Albini, “Inflammation, inflammatory cells and angiogenesis: decisions and indecisions,” Cancer and Metastasis Reviews, vol. 27, no. 1, pp. 31–40, 2008.
[28] D. Hanahan and R. A. Weinberg, “Hallmarks of cancer: the next generation,” Cell, vol. 144, no. 5, pp. 646–674, 2011.
[29] A. Bruno, G. Ferlazzo, A. Albini, and D. M. Noonan, “A think tank of TINK/TANKs: tumor-infiltrating/tumor-associated natural killer cells in tumor progression and angiogenesis,” Journal of the National Cancer Institute, vol. 106, no. 8, article dju200, 2014.
[30] A. Bruno, C. Focaccetti, A. Pagani et al., “The proangiogenic phenotype of natural killer cells in patients with non-small cell lung cancer,” Neoplasia, vol. 15, no. 2, pp. 133–142, 2013.
[31] J. Baginska, E. Viry, J. Pagetti et al., “The critical role of the tumor microenvironment in shaping natural killer cell-mediated anti-tumor immunity,” Frontiers in Immunology, vol. 4, article 490, 2013.
[32] R. A. Flavell, S. Sanjabi, S. H. Wrzesinski, and P. Licona-Limón, “The polarization of immune cells in the tumour environment by TGFbeta,” Nature Reviews Immunology, vol. 10, no. 8, pp. 554–567, 2010.
[33] H. Ikushima and K. Miyazono, “TGFβ 2 signalling: a complex web in cancer progression,” Nature Reviews Cancer, vol. 10, no. 6, pp. 415–424, 2010.
[34] L. Yang, Y. Pang, and H. L. Moses, “TGF-β and immune cells: an important regulatory axis in the tumor microenvironment and
progression,” *Trends in Immunology*, vol. 31, no. 6, pp. 220–227, 2010.

[35] D. S. Allan, B. Rybalov, G. Awong et al., “TGF-beta affects development and differentiation of human natural killer cell subsets,” *European Journal of Immunology*, vol. 40, no. 8, pp. 2289–2295, 2010.

[36] A. S. Cerdeira, A. Rajakumar, C. M. Royle et al., “Conversion of peripheral blood NK cells to a decidual NK-like phenotype by a cocktail of defined factors,” *Journal of Immunology*, vol. 190, no. 8, pp. 3939–3948, 2013.

[37] D. B. Keskin, D. S. J. Allan, B. Rybalov et al., “TGFβ promotes conversion of CD16+ peripheral blood NK cells into CD16– NK cells with similarities to decidual NK cells,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 9, pp. 3378–3383, 2007.

[38] K. D. Beaman, M. K. Jaiswal, G. K. Katara et al., “Pregnancy is a model for tumors, not transplantation,” *American Journal of Reproductive Immunology*, vol. 76, no. 1, pp. 3–7, 2016.

[39] S. G. Holtan, D. J. Creedon, P. Haluska, and S. N. Markovic, “Neutrophils in type 1 diabetes: a disease of developmental origins,” *Pediatric Diabetes*, vol. 10, no. 1, pp. 33–37, 2009.

[40] D. W. Hoskin, J. S. Mader, S. J. Furlong, D. M. Conrad, and J. Blay, “Inhibition of T cell and natural killer cell function by adenosine and its contribution to immune evasion by tumor cells (Review),” *International Journal of Oncology*, vol. 32, no. 3, pp. 527–535, 2008.

[41] V. Kumar and A. Sharma, “Adenosine: an endogenous modulator of innate immune system with therapeutic potential,” *European Journal of Pharmacology*, vol. 616, no. 1–3, pp. 7–15, 2009.

[42] G. Berchem, M. Z. Noman, M. Bossel et al., “Hypoxic tumor-derived microvesicles negatively regulate NK cell function by a mechanism involving TGF-beta and miR23a transfer,” *Oncoimmunology*, vol. 5, no. 4, Article ID e1062968, 2016.

[43] L. Antoniolii, C. Blandizzi, P. Pacher, and G. Haskó, “Immunity, inflammation and cancer: a leading role for adenosine,” *Nature Reviews Cancer*, vol. 13, no. 12, pp. 842–857, 2013.

[44] D. W. Greening, S. K. Gopal, R. Xu, R. J. Simpson, and W. Chen, “Exosomes and their roles in immune regulation and cancer,” *Seminars in Cell and Developmental Biology*, vol. 40, pp. 72–81, 2015.

[45] J. Webber, V. Yeung, and A. Clayton, “Extracellular vesicles as modulators of the cancer microenvironment,” *Seminars in Cell and Developmental Biology*, vol. 40, pp. 27–34, 2015.

[46] K. Denzer, M. J. Kleijmeer, H. F. G. Heijnen, W. Stoorvogel, and H. J. Geuze, “Exosome: from internal vesicle of the multivesicular body to intercellular signaling device,” *Journal of Cell Science*, vol. 113, no. 19, pp. 3365–3374, 2000.

[47] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee, and J. O. Lötvall, “Exosome-mediated transfer of miRNAs and microRNAs is a novel mechanism of genetic exchange between cells,” *Nature Cell Biology*, vol. 9, no. 6, pp. 654–659, 2007.

[48] A. Clayton, J. P. Mitchell, J. Court, S. Linnane, M. D. Mason, and Z. Tabi, “Human tumor-derived exosomes down-regulate NKG2D expression,” *Journal of Immunology*, vol. 180, no. 11, pp. 7249–7258, 2008.

[49] A. Clayton and Z. Tabi, “Exosomes and the MICA-NKG2D system in cancer,” *Blood Cells, Molecules, and Diseases*, vol. 34, no. 3, pp. 206–213, 2005.

[50] L. Muller, M. Mitsuhashi, P. Simms, W. E. Gooding, and T. L. Whiteside, “Tumor-derived exosomes regulate expression of immune function-related genes in human T cell subsets,” *Scientific Reports*, vol. 6, article 20254, 2016.

[51] D. Gotthardt, E. M. Putz, E. Grundsober et al., “STAT5 is a key regulator in NK cells and acts as a molecular switch from tumor surveillance to tumor promotion,” *Cancer Discovery*, vol. 6, no. 4, pp. 414–429, 2016.

[52] J. E. Phillips, J. J. Couper, M. A. S. Penno et al., “Type 1 diabetes: a disease of developmental origins,” *Pediatric Diabetes*, 2016.

[53] J. Huang, Y. Xiao, A. Xu, and Z. Zhou, “Neutrophils in type 1 diabetes,” *Journal of Diabetes Investigation*, vol. 7, no. 5, pp. 652–663, 2016.

[54] N. Van Gassen, W. Staels, E. Van Overmeire et al., “Concise review: macrophages: versatile gatekeepers during pancreatic beta-cell development, injury, and regeneration,” *Stem Cells Translational Medicine*, vol. 4, no. 6, pp. 555–563, 2015.

[55] D. H. Wagner, “Of the multiple mechanisms leading to type 1 diabetes, T cell receptor revision may play a prominent role (is type 1 diabetes more than a single disease?),” *Clinical & Experimental Immunology*, vol. 185, no. 3, pp. 271–280, 2016.

[56] G. S. Eisenbarth, “Type 1 diabetes mellitus. A chronic autoimmune disease,” *New England Journal of Medicine*, vol. 314, no. 21, pp. 1360–1368, 1986.

[57] M. A. Kelly, M. L. Rayner, C. H. Mijovic, and A. H. Barnett, “Molecular aspects of type 1 diabetes,” *Molecular Pathology*, vol. 56, no. 1, pp. 1–10, 2003.

[58] A. G. Baxter and M. J. Smyth, “The role of NK cells in autoimmune disease,” *Autoimmunity*, vol. 35, no. 1, pp. 1–14, 2002.

[59] M. Flodstrom, A. Maday, D. Balakrishna, M. M. Cleary, A. Yoshimura, and N. Sarvetnick, “Target cell defense prevents the development of diabetes after viral infection,” *Nature Immunology*, vol. 3, no. 4, pp. 373–382, 2002.

[60] L. Poirot, C. Benoist, and D. Mathis, “Natural killer cells distinguish innocuous and destructive forms of pancreatic islet autoimmune,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 21, pp. 8102–8107, 2004.

[61] I. Lee, H. Qin, J. Trudeau, J. Dutz, and R. Tan, “Regulation of autoimmune diabetes by complete Freund’s adjuvant is mediated by NK cells,” *Journal of Immunology*, vol. 172, no. 2, pp. 937–942, 2004.

[62] L. D. Poulton, M. J. Smyth, C. G. Hawke et al., “Cytometric and functional analyses of NK and NKT cell deficiencies in NOD mice,” *International Immunology*, vol. 13, no. 7, pp. 887–896, 2001.

[63] S. E. Johansson, H. Hall, J. Bjorklund, and P. Höglund, “Broadly impaired NK cell function in non-obese diabetic mice is partially restored by NK cell activation in vivo and by IL-12/IL-18 in vitro,” *International Immunology*, vol. 16, no. 1, pp. 1–11, 2004.

[64] M. J. Hussain, L. Alviggi, B. A. Millward, R. D. G. Leslie, D. A. Pyke, and D. Vergani, “Evidence that the reduced number of natural killer cells in Type 1 (insulin-dependent) diabetes may be genetically determined,” *Diabetologia*, vol. 30, no. 12, pp. 907–911, 1987.
[98] A. Selathurai, V. Deswaerte, P. Kanellakis et al., “Natural killer (NK) cells augment atherosclerosis by cytotoxic-dependent mechanisms,” Cardiovascular Research, vol. 102, no. 1, pp. 128–137, 2014.

[99] M. F. Linton, A. S. Major, and S. Fazio, “Proatherogenic role for NK cells revealed,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 24, no. 6, pp. 992–994, 2004.

[100] K. Dong, J. Ge, S. Gu et al., “Ox-LDL can enhance the interaction of mice natural killer cells and dendritic cells via the CD48-2B4 pathway,” Heart and Vessels, vol. 26, no. 6, pp. 637–645, 2011.

[101] F. Cheng, L. Twardowski, K. Reifenberg et al., “Combined B, T and NK cell deficiency accelerates atherosclerosis in BALB/c mice,” PLoS One, vol. 11, no. 8, Article ID e0157311, 2016.

[102] I. Bonaccorsi, C. De Pasquale, S. Campana et al., “Natural killer cells in the innate immunity network of atherosclerosis,” Immunology Letters, vol. 168, no. 1, pp. 51–57, 2015.

[103] K. Kotfis, J. Biernawska, M. Zegan-Baranska, and M. Zukowski, "Peripheral Blood Lymphocyte Subsets (CD4+, CD8+ T Cells, NK Cells) in Patients with Cardiovascular and Neurological Complications after Carotid Endarterectomy," International Journal of Molecular Sciences, vol. 16, no. 5, pp. 10077–10094, 2015.

[104] H. Bruunsgaard, A. N. Pedersen, M. Schroll, P. Skinhoj, and B. K. Pedersen, “Decreased natural killer cell activity is associated with atherosclerosis in elderly humans,” Experimental Gerontology, vol. 37, no. 1, pp. 127–136, 2001.

[105] L. Jonasson, K. Backteman, and J. Ernerudh, “Loss of natural killer cell activity in patients with coronary artery disease,” Atherosclerosis, vol. 183, no. 2, pp. 316–321, 2005.