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Review Article

Biochemistry Section

Therapeutic Applications: Natural Killer Cells

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ABSTRACT

Natural Killer (NK) cells are potent cytotoxic effector cells for cancer therapy and also for keeping severe viral infections in check. Since, they represent only 10% of the lymphocytes and are often dysfunctional, there are technical challenges in obtaining sufficient numbers of functionally active NK cells from a patient's blood. Recent advances in therapeutic regimen of NK cells are intended to enhance NK cell activity and targeting strategies. With recent advances in the field of NK cell biology and translational research, it seems that over the next few years, NK cell immunotherapy will move to the forefront of cancer immunotherapy. The current review focuses on recent developments in NK cell immunotherapy including various approaches i.e. augmentation of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC), manipulation of receptor-mediated activation, and adoptive immunotherapy with ex vivo-expanded NK cells, Chimeric Antigen Receptor (CAR)-engineered, or engager-modified NK cells.

Keywords: Adoptive immunotherapy, Chimeric antigen receptor, Immunosurveillance

INTRODUCTION

Cytotoxicity via innate immune response against various malignancies, including leukaemia, is contributed by NK cells and this quality of these cells to produce antitumor effect is a subject of intense investigation in the field of cancer immunotherapy. Discovery dates back to 40 years [1], new and exciting area of NK cells biology continue to emerge to make its effective use to treat cancer. A thorough understanding of NK cell functions, its multitude of receptor functions, its role in tumour surveillance will surely help to develop novel, more efficient therapies based on these cells to check tumours. NK cells are long-lived and harnessing these cells for treating neoplastic growth, actually evolved nearly 30 years back for unleashing autologous NK cells cytotoxicity by exogenous cytokines and other activators to adoptively transfer autologous or allogeneic NK cells [2]. In the year 1980, cellular products of autologous NK cells were pioneered by the National Cancer Institute to treat cancer [3]. All these strategies led to the emergence of adoptive transfer of autologous or allogeneic NK cells. It is evident from various clinical observations that following allogeneic stem cell transplantation, NK cells rapidly proliferate and if haploidentical donor and recipients are mismatched in KIR-KIR ligands due to class 1 HLA, the donor NK cells mediate strong antileukaemic cellular response preventing patient from leukaemia relapses [4,5]. Besides adoptive immunotherapy with ex vivo-expanded treatment, augmentation of ADCC, manipulation of receptor-mediated activation, and CAR-engineered or engager-modified NK cells are few more novel approaches which pave the way for successfully implementing the NK cells as therapeutic agents. Donor NK cells do not attack non-haematopoietic tissues, and that makes an NK-mediated antitumor effect in the absence of Graft-Vs-Host Disease (GVHD) [6]. Manipulation of the NK cell product, host factors, and tumour targets are the subject of intense research in order to enhance therapeutic benefits of NK cell based immunotherapy [7]. Expression of CARs in order to redirect antitumor activity via genetic engineering [8] is one of the significant developments in the field with promising results. Mature NK cells have been chosen as an attractive candidate effector cells to express CARs for adoptive immunotherapies as they have short lifespan and potent cytolytic function. Manipulation of NK cells cytolytic activity via creating bispecific or trispecific antibodies to augment the activity against tumour associated antigens is another innovative approach.

NK Cells and Adoptive Immunotherapy

Human NK cells are entirely dependent on Killer-cell Immunoglobulin-like Receptor (KIR) complex for their functions, which are expressed over the surface of cells [9]. They are meant to interact with MHC1 present over the cells leading to generation of inhibitory signals in most circumstances but NK cell functions get activated when they are exposed to tumour cells lacking MHC1 [9]. Activating NK cell receptors are NKP30, NKP44, NK46, NKG2D, DNAM-1 which is constitutively expressed on all NK cells [10]. There are few protein molecules expressed on the cells to actively lure receptors of NK cells. They are HLA class 1 related MICA & MICB, CD112 (Nectin-2), class I like cytomegalovirus-homologous ULBP proteins, the ligand CD155 (polio virus receptor) mediating NK cells killing [10,11]. The tumour cells having MHC one and/or lacking ligands whose signal activate NK cell receptors, escape killing by NK cells [12]. NK cells incubation with cytokines particularly IL-2, or IL-15 increase its killing capacity broadly and cultured tumour cells normally insensitive to NK cells become susceptible to lysis. Moreover, cytokines that activate NK cells are synthesised with monoclonal antibodies against resistant cell lines in vitro and in mouse xenograft model [13]. On the basis of various studies done in this direction led to the conclusion that IL-2 can be safely administered to induce NK cells [13]. It may cause disproportionate increase in NK cells. With advancement in knowledge about KIR and evolving understanding of NK cells licensing and the role that HLA plays in the process, led to switching to allogeneic NK cell transport as possible alternate. Ruggeri L et al., have reported that NK cell cytotoxicity is enhanced if KIR-HLA class I mismatch happens, in clinical trials using allogeneic T cell depleted haematopoietic cell transplantation from haploidentical donors in patients with amyloid leukaemia. Allogeneic donor derived NK cells were not associated with GVHD. They showed remarkably potent antileukaemic response. Therefore, it was being hypothesised that mature haploidentical NK cells alone without stem cell transplantation could represent a promising tool as antitumor therapy [14,15].

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Modification of Fc fragment of antibody to trigger NK cell mediated ADCC is one of the effective ways to augment the therapeutic efficacy of monoclonal antibodies (mAb) [9]. Through Fcγ receptor CD16, NK cells mediate ADCC, and this receptor is expressed from

CD56dim subset. Viable modifications in its expression may lead to a novel strategy to augment NK cell effector functions [16]. Few clinically approved antibodies, for example, rituximab or cetuximab, target tumour associated antigens that triggers NK cell mediated ADCC partially [17]. Several studies done on mouse model have shown that for efficacy of mAb, efficient FcR (Fc Receptor) interactions are required [18]. Romain G et al., have reported the successful engineering of Fc region of IgG mAb, HuM195 targeting the Acute Myelogenous Leukaemia (AML) antigen CD33, via triple mutation S293D/A330L/I332 (DLE) and using timelapse imaging microscopy in nanowell grid (TIMING, the method to analyse kinetics of thousands of NK cells and mAb coated targets). It was demonstrated that these DLE-HuM195 antibodies increased both the quantity and quality of NK cell mediated ADCC via recruitment of NK cells to cause cytotoxicity through CD16 signals [17-19]. One more approach was reported including investigation in order to enhance NK cell mediated ADCC involving antibody engineering and therapeutic combination for synergising effects. For example, mogamulizumab successfully induced ADCC activity against CCR4-positive cell line, inhibiting the growth of Epstein-Barr virus (EBV) positive NK cells lymphomas [16]. Obinutuzumab (GA101) is another novel mAb which is glycoengineered against CD20 with increased FcγRIII binding and ADCC activity [16]. Activation caused by obinutuzumab happens in a manner so as to be not affected by KIR/HLA interactions. In most of the cases where mAb is non-engineered (i.e., rituximab), the NK cell mediated cytotoxicity is under the jurisdiction of KIR mediated inhibition. In another approach, ADCC response has been potentiated in vitro in the presence of mAb that block KIR/HLA1interaction. In fact, a fully humanised anti-KIR mAb 1-7Fa (IPH2101) with ability to block KIR DS1/S2 and KIR L1/L2/L3 was generated [16]. This anti-KIR mAb was found to augment NK cell mediated lysis of HLA-C expressing tumour cells, including autologous AML blast and autologous CD138+ multiple myeloma cells in vitro. Moreover, in the dose escalation phase 1 clinical trial, these mAb were reported to be safe and could block KIRs for a long time [16]. Findings reported by Lin W et al., suggest that agonistic cells targeted mAb lead to expression of activation marker CD137 following FcR triggering during ADCC. This agonistic mAb targeting CD137 augments the NK cell activity including degranulation, IFN-γ secretion and antitumour cytotoxicity in vitro and in vivo in preclinical models of tumours [20]. Anti CD137 antibody with retuximab combination is in phase I trial in lymphoma patients. In fact, a number of combinations have been designed to boost ADCC and are showing promising results in various stages of clinical trials. Further studies are needed to be done in search of some novel therapeutic substitute for cancer.

Human NK Cell Lines as Source of NK Cell Immunotherapy

Adoptive transfer of NK cell lines is far superior over the patient and donor derived NK cells, due to lack of immunogenicity, wide expansion capacity and 'off the shelf' availability of the cell lines. In the literature, various human NK cell lines e.g., NK-92, KHYG-1, have been well documented that exhibit an effective spectrum both in clinical and preclinical settings [21]. The cytotoxic cell lines have been created from patients from clonal NK cell lymphoma, followed by their expansion in culture medium via IL-2 [21]. Out of the six known NK cell lines, NK-92 cell line is the most efficient and exhibits high antitumour cytotoxicity with consistent reproducibility. It can easily be genetically modulated to recognise specific neoplastic antigens. It can also augment mAb activity through ADCC. Best clinical effects and minimal side-effects have been observed when NK-92 cell line products were infused in patients with advanced cancer [16,21].

CAR Modified NK Cells

The CAR has been known to redirect the T-cell specificity against leukaemia. Patients suffering from lymphoblastic leukaemia were transduced with CD19-BB-ζ receptors and dramatic clinical responses were observed. NK cells with small lifespan and potent cytolytic characteristics are proved to be attractive and suitable candidate effector cells to express CARs [16,22]. They provide an excellent source of 'off the shelf' cellular therapy for cancer. It has been possible to genetically engineer NK cells to express CARs. Primary NK cells as NK-92 cells were successfully modulated to express CARs via genetic engineering which can work against a number of targets i.e., CD19, CD20, CD244, HER2. CAR transduced NK cells have shown excellent results in the in vitro and in vivo killing of tumour cells [23]. Shimasaki N et al., have expressed receptors containing CD3 ζ and 4-1 BB signalling molecules (anti-CD19-BB-ζ) in human NK cells after mRNA electroporation using clinical grade electroporator. Adequate transfection efficiency was observed 24 hours after electroporation, with media anti-CD-19 BB-ζ expression of 40.3% in freshly purified and 61.3% in expanded cells. These transfected NK cells with considerable cytotoxicity were tested in xenograft model of B-cell leukaemia. Another way can be the development of CAR modified NK cells that target NKG2D ligand present on the surface of tumour cells, rendering it more susceptible to a variety of haematolytic and solid malignancy. Well targeted NK cells targeting ganglioside GD2 present on neuroblastoma have been tested in the preclinical setting. GD2 has also been found to be expressed by breast cancer cells [15,24,25]. So the NK-CAR combination can also be tested for its potential effects. CAR expressing NK-92 cells may offer a very efficient source of NK cell based immunotherapeutic trials. NK-92 cells have vast capacity to expand easily. A number of factors can affect the activation, anti-tumour activity and persistence of CAR-NK cells. Second and third generation of CAR construct incorporating additional domains (e.g., CD28, OX-40, or 4-1BB) showed enhanced activation both in vitro and in vivo, and the persistence of CAR T cells [26]. There are many concerns including on-target/off-target effects, GVHD, cytokine release syndrome, tumour lysis syndrome, toxicity to normal tissues due to limited selectivity of target antigen, thereby requiring further studies to equip CAR modified NK cells with safety switches or suicide genes.

Bispecific and Trispecific Engagers to NK Cell Therapy

In one more novel approach, in order to redirect NK cell cytotoxicity towards tumour cells, bispecific and trispecific antibodies have been developed, called BiKE and TriKE, by genetic engineering. BiKEs are constructed by joining a single chain Fv region of immunoglobulin against CD16 and a single chain Fv against CD16 and a single chain Fv against a tumour associated antigen (in BiKE) and two tumour associated antigens (in TriKE) [27]. It was reported by Gleason MK et al., that bispecific (bscFv) CD16/CD19 and trispecific (tscFv) CD16/CD19/CD22 engagers directly trigger NK cell activation through CD16 [28]. In this way, the cytolytic activity of cytokines production of NK cells is triggered. CD16*33BiKE in refractory AML were also tested and developed by the same group of workers. They demonstrated that potent killing by NK cells can overcome the inhibitory effects of KIR signalling.

CONCLUSION

A number of clinical evidence based studies have been conducted, including mostly manipulated NK cell products. Their ultimate goal is to acquire maximum therapeutic benefits of NK cell immunotherapy. In the current scenario, success of NK cell expansion interventions remains unpredictable, particularly for solid tumours. These tumours induce immunosuppressive microenvironment that inhibits the immune response. In fact, in different types of tumours and patient population, the tumour products and cytokine impacts are different. Future trials will be designed in a manner so as to overcome host immune barrier of NK antitumour reactivity. Enormous progress has been made in last four decades since the discovery of NK cells by Dr.

Herberman and his co-workers. Current research is actively being pursued to progressively translate this NK cells based therapeutic approach into a promising one for successfully treating various malignancies.

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