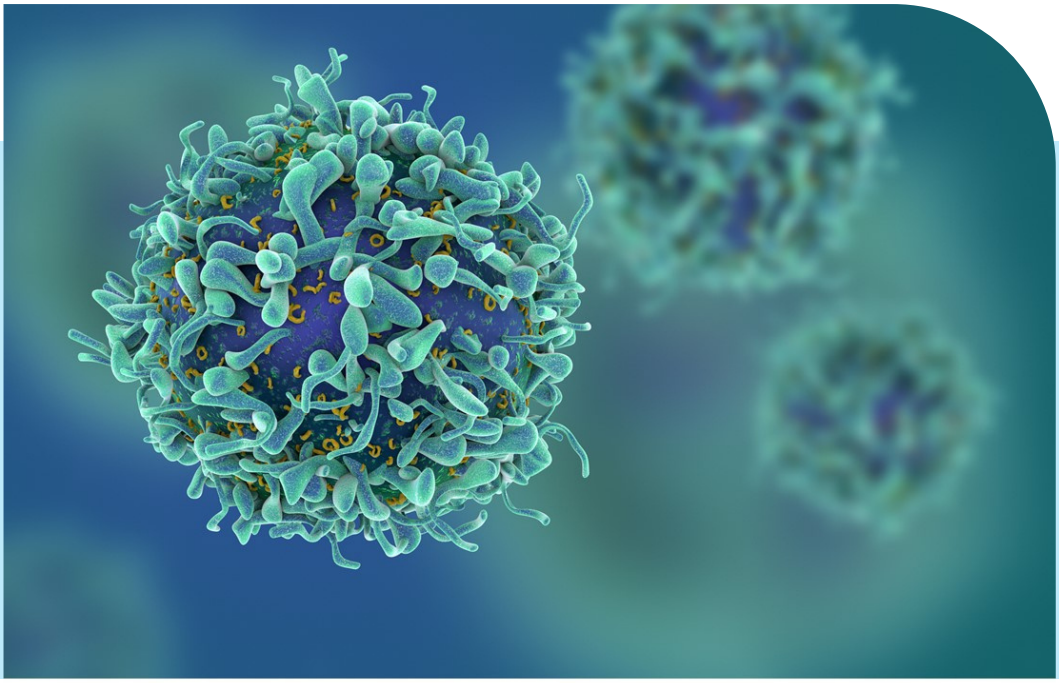
Regenmedlab

Leading Regenerative Medicine Innovation



Immune Cell Manufacturing

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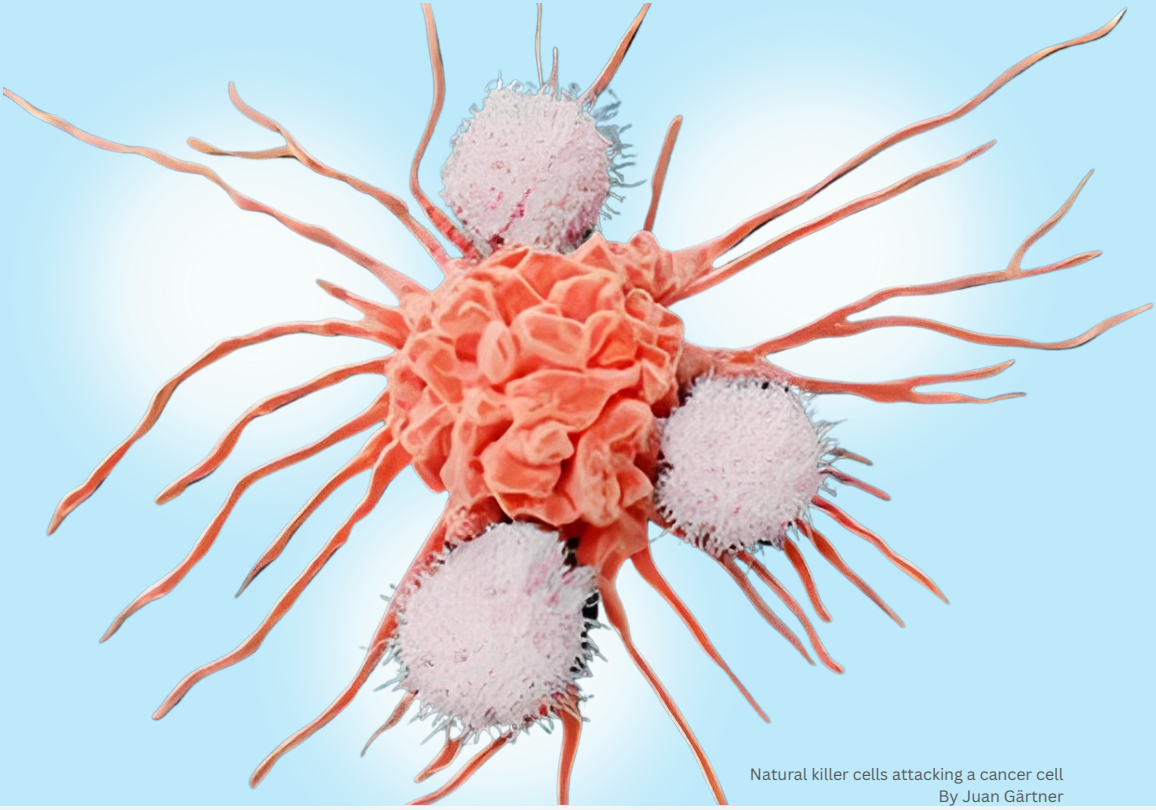
CHAPTER 1

Technology of isolating and expanding Natural killer cell (NK)

Natural killer cell

In 1975, Rolf Keissling and his colleagues in Sweden made a significant discovery regarding the cytotoxic capabilities of natural cells. They identified a specific type of immune cell that has the ability to effectively kill various types of tumor cells. As a result of this observation, Keissling named these cells "natural killer cells" (NK cells). NK cells make up approximately 5-20% of circulating cells in the human body. They exhibit rapid responses to virus-infected cells (often within three days of infection) as well as to the formation of tumors. When immune cells encounter cells infected with viruses, they are able to detect antigens presented within the MHC complex of the infected cells. Following this detection, immune cells initiate a response by releasing cytokines, leading to the death of the infected cells through apoptosis or cell lysis.

What sets NK cells apart is their unique ability to identify and eliminate stressed cells in the absence of antibodies and MHC. This allows them to respond swiftly to disease-causing agents. It is this characteristic that earned them the name "natural killers," as they do not require additional activation signals to eliminate cells with MHC class 1 defects. This is particularly crucial because immune cells (such as T lymphocytes) often fail to recognize and eliminate harmful cells defected in MHC 1.



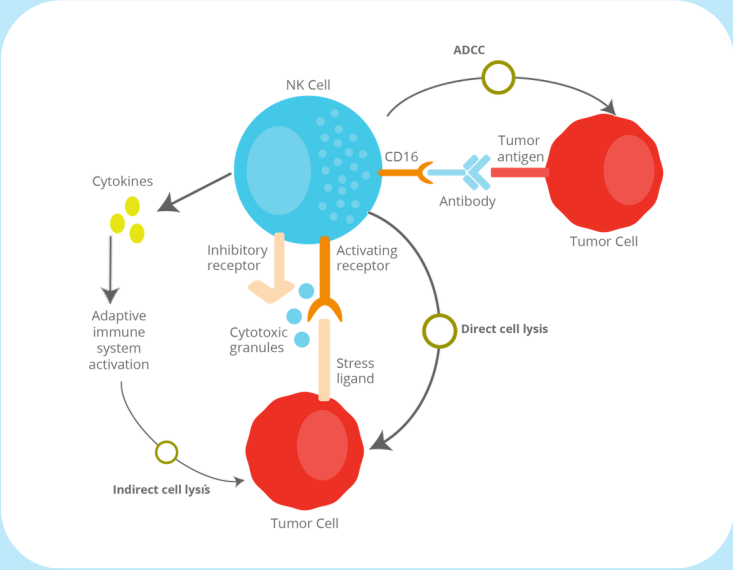
Natural killer cells attacking a cancer cell
By Juan Gärtner

NK cells are characterized by the presence of the CD56 molecule and the absence of CD3 on their cell surface. The typical immune profile of NK cells is $CD56^{+}CD3^{-}$. NK cells with the $CD56^{+}CD3^{-}$ profile are referred to as classic NK cells, distinguished from another type of NK cell known as NKT cells. NKT cells express the $CD56^{+}CD3^{+}$ surface marker, indicating that they express the T cell antigen receptor (TCR), unlike NK cells.

Within the NK cell population, scientists have identified two subsets based on the level of CD56 expression: CD56bright and CD56dim. CD56brightCD3- NK cells are known to function similarly to T helper cells, primarily exerting their effects through the release of cytokines. In contrast, CD56dimCD3- NK cells can induce cell toxicity through a mechanism called antibody-dependent cellular cytotoxicity (ADCC).

In general, after NK cells detect infected or abnormal cells, they eliminate tumor cells through several mechanisms:

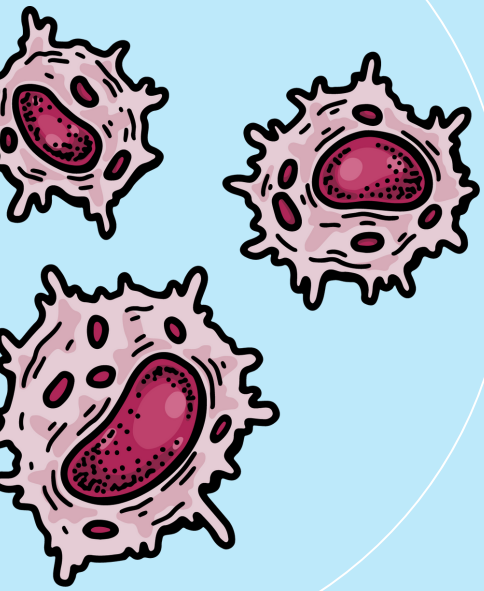
- Directly eliminating target cells by releasing toxic granules containing granzyme and perforin.
- Indirectly eliminating target cells by secreting cytokines and chemokines, such as pro-inflammatory cytokines, to facilitate the recognition of target cells.
- Cell toxicity mechanisms can also depend on antibodies.



Kill target cells mechanism of NK cell

The cellular therapy known as natural killer cell (NK) infusion, or NK cell therapy, has been utilized in numerous recent clinical trials. In general, this therapy is used for two purposes:

1. NK cell infusion in cancer treatment and prevention of cancer recurrence.
2. NK cell infusion for immune restoration and enhancement, particularly in cases of aging.



Technology of isolating and expanding Natural killer cell (NK)

In order to meet the demand for isolating and expanding a large quantity of NK cells for research and clinical trials, Regenmedlab has introduced the NKCult cellular therapy kit.

NKCult is a comprehensive set of chemicals and materials specifically designed for the isolation and expansion of NK cells from peripheral blood. With NKCult, users have all the necessary tools and chemicals for blood collection, mononuclear cell isolation, as well as culture, and expansion of NK cells. This eliminates the need for additional chemicals or materials, making the process more convenient.

Unlike other methods, NKCult does not require the use of feeder cells or coating, simplifying the procedure and reducing the associated risks. This ensures a more stable and consistent process for isolating and expanding NK cells. Additionally, NKCult is free from animal-derived proteins, eliminating the need for supplementary components before use.

Overall, NKCult offers a streamlined and efficient solution for isolating and expanding NK cells, making it a valuable resource for researchers and clinicians alike.

Product	Cat. No	Packing specification	Blood volume	NK cell yield*	Degree of purity
NKCult Mini	348	Kit	7.5 mL	1–2 billion cells	> 50 %
NKCult	109	Kit	15 mL	2– 4 billion cells	> 50 %
NKCult Max	349	Kit	30 mL	4–8 billion cells	> 50 %
NKCult Pure Mini	350	Kit	7.5 mL	1–2 billion cells	> 90 %
NKCult Pure	351	Kit	15 mL	2–4 billion cells	> 90 %
NKCult Pure Max	352	Kit	30 mL	4–8 billion cells	> 90 %

**After 15 days of cultivation, the amount of cell harvested depends on each patient*
***The purity of NK cell was determined based on percentage of CD56 expression using flow cytometry*

The NKCult kit is designed to accommodate two different groups:

Kits with moderate purity (> 50%): These kits are designed with simplified steps, excluding the removal of T cells ($CD3^+$ cells) before culturing. In this kit, a selective and enriching medium for NK cells is used, and the purity of the harvested NK cells ranges from 50% to 80%. This group includes three different sizes for different blood volumes: 7.5 mL, 15 mL, and 30 mL.

Kits with high purity (> 90%): These kits are designed with an additional step to remove T cells before the selective culture and expansion of NK cells. This allows for a higher purity of NK cells (> 90%) after harvesting. Similarly, this group includes three different sizes for different blood volumes: 7.5 mL, 15 mL, and 30 mL.

The NKCult kit offers flexibility in choosing the desired purity level, providing researchers and clinicians with options to suit their specific needs.

CHAPTER 2

Technology of isolating and expanding Cytokine-induced killer cell (CIK)

Cytokine-induced killer cell (CIK)

Cytokine-induced killer (CIK) cells are a unique group of immune cells that exhibit properties of both T cells and natural killer (NK) cells. These cells are generated in the laboratory by stimulating mononuclear cells (MNCs) derived from peripheral blood or umbilical cord blood with specific cytokines. The discovery of CIK cells dates back to 1991, credited to G.H. Schmidt-Wolf, and their first successful application in cancer treatment in humans was reported in 1999 by the same researcher.

Thanks to their dual functionality resembling both T cells and NK cells, CIK cells possess the ability to target and eliminate cancer cells using the mechanisms of both types of lymphocytes. This means they can recognize and destroy cancer cells either through the conventional major histocompatibility complex (MHC) recognition pathway or by bypassing MHC restrictions.

Over the years, CIK cells have been extensively researched and proven to be effective in cancer therapy. The positive outcomes from numerous clinical trials conducted in the past decade demonstrate that CIK cells can efficiently eradicate cancer cells with minimal side effects. In a comprehensive review by Zhang and Schmidt-Wolf (2020) on 10 years of CIK immunotherapy for cancer treatment, it was revealed that there were 106 registered clinical trials involving 10,225 cancer patients on the International Registry of Clinical Trials (IRCC) as of 2020. The results showcased a notable improvement in both average disease-free survival rates and overall survival rates in 27 studies, with 9 studies specifically reporting significant enhancements in survival rates after 5 years.

Technology of isolating and expanding Cytokine-induced killer cell (CIK)

CIKCult is a technology developed by Regenmedlab that enables the isolation of mononuclear cells (MNCs) from peripheral blood, induction of CIK cells, and expansion of CIK cells. CIKCult is a complete set of chemicals and materials that users can conveniently utilize for production without the need for additional preparation. The product has been carefully formulated to cater to three different volumes of collected blood: 7.5 mL, 15 mL, and 30 mL. Depending on the volume of collected blood, the quantity of harvested cells after 15 days varies, ranging from 1-8 billion cells. The harvested CIK cell population will contain a subset of $CD56^{+}CD3^{+}$ cells, comprising approximately 20-40% of the total cell population.

Product	Cat. No	Packing specification	Blood volume	CIK cell yield*	$CD3^{+}CD56^{+}$ cell population
CIKCult Mini	346	Kit	7.5 mL	1–2 billion cells	> 20 %
CIKCult	103	Kit	15 mL	2–4 billion cells	> 20 %
CIKCult Max	347	Kit	30 mL	4–8 billion cells	> 20 %

**The amount of cells obtained after 15 days of culture, the output depends on each patient*

CHAPTER 3

Technology of isolating, selecting, and expanding gamma-delta T cell

Gamma-delta T cell

Gamma-delta T cells are a unique subset of T cells that display a distinct gamma-delta T cell receptor (TCR) on their surface. Unlike the majority of T cells in the human body, which express an alpha-beta TCR composed of alpha and beta glycoprotein chains, gamma-delta T cells possess a TCR structure with gamma and delta chains. These gamma-delta T cells are relatively less abundant compared to alpha-beta T cells.

One notable feature of gamma-delta T cells is their ability to recognize and target antigens independently of the major histocompatibility complex (MHC) molecules on antigen-presenting cells. This gives them the extraordinary capacity to eliminate target cells that may not be presented to conventional T cells.

Due to their unrestricted antigen recognition and potent cytokine secretion, gamma-delta T cells have been extensively studied and employed in cancer immunotherapy. These cells demonstrate dual functions in immune responses:

1. Effector function: Gamma-delta T cells can interact with specific molecules on the surface of cancer cells, triggering the release of toxic substances and pro-inflammatory cytokines. They can also activate other immune cells involved in anti-tumor responses. Additionally, gamma-delta T cells can directly destroy target cells via antibody-dependent cell-mediated cytotoxicity (ADCC) mechanisms. Furthermore, they secrete potent immune-regulatory molecules like interferon-gamma and IL-17, which enhance the expression of major histocompatibility complex class I molecules. This modulation positively influences the activity of cytotoxic T cells and promotes anti-tumor immunity. Gamma-delta T cells can also interact with dendritic cells, contributing to the development of Th1 immune responses.
2. Regulatory function: Gamma-delta T cells exert regulatory functions by influencing the tumor microenvironment. They can modulate the expression of key factors involved in immune regulation, such as FoxP3 and Helios transcription factors. By regulating the interaction between CD86 on antigen-presenting cells and CTLA-4 on gamma-delta T cells, they can impact the function of other immune cells like CD, NK, iNKT, and CD8 lymphocytes.

In summary, the distinct characteristics and dual functionality of gamma-delta T cells make them valuable candidates for cancer immunotherapy and highlight their potential in modulating immune responses for therapeutic purposes.

Technology of isolating, selecting, and expanding gamma-delta T cell

Regenmedlab is proud to introduce the γδCult product range, offering a streamlined workflow, reduced risk of contamination, and improved replicability. With γδCult, you have everything you need - from tools to chemicals - specifically designed for peripheral blood samples of 7.5 mL, 15 mL, and 30 mL. Over the course of 15 days, this exceptional system yields a substantial and robust cell harvest.

Our products are free from animal-derived proteins, eliminating the need for supplementary components before use... Moreover, the initial 48-hour culture medium is fortified with antibiotics and antifungals, effectively quelling any potential pathogens and ensuring a clean working environment.

Product	Cat. No	Packing specification	Blood volume	γδT cell population
γδCult Mini	353	Kit	7.5 mL	> 90 %
γδCult	354	Kit	15 mL	> 90 %
γδCult Max	355	Kit	30 mL	> 90 %

CHAPTER 4

Technology of isolating and expanding Dendritic cell

Dendritic cell

Dendritic cells, integral to the immune cell system, originate from hematopoietic stem cells found in the bone marrow. These cells can be derived either from CD14+ mononuclear cells or CD34+ precursor cells. With in vitro conditions, it is feasible to generate a substantial quantity of dendritic cells for research purposes and therapeutic interventions. One commonly employed method involves culturing progenitor cells from the bone marrow or mononuclear cells with GM-CSF and IL-4.

The pioneering contribution of Ralph M. Steinman led to the initial discovery of dendritic cells in 1973, an achievement that earned him the Nobel Prize in 2011. The advancements in dendritic cell therapy have paved the way for its safe and viable application in cancer treatment.

Technology of isolating and expanding Dendritic cell

The technology of isolating and expanding dendritic cells from human peripheral blood was initially researched by Dr. Pham Van Phuc in 2009. Over the course of nearly 15 years, this technology has undergone significant advancements, simplifying procedural steps and enhancing the efficacy of generating mature dendritic cells. The DCCult product line, derived from this innovative technology, encompasses all the necessary materials and chemicals essential for efficient blood collection, mononuclear cell isolation, and successful induction of mononuclear cells into mature dendritic cells. Notably, the DCCult culture medium is completely devoid of animal-derived proteins, making it a comprehensive and optimal choice for direct cell cultivation.

Product	Cat. No	Packing specification	Blood volume	Dendritic cell population
DCCult Mini	371	Kit	7,5 mL	> 70 %
DCCult	372	Kit	15 mL	> 70 %
DCCult Max	373	Kit	30 mL	> 70 %

CHAPTER 5

Technology of immune cell cryopreservation

DMSO is known to be toxic and inhibits the proliferation and cytokine secretion of immune cells. Therefore, it is important to use DMSO in preservation solutions at low concentrations. Regenmedlab offers two freezing media for immune cell cryopreservation: ImmuCryosave technology, which contains 5% DMSO, and ImmuCryosave OTS TH technology, which is DMSO-free. Both of these media are formulated with high-quality, injectable-grade ingredients, ensuring safety. They have been proven to support high cell survival rates post-thaw, with over 90% of cells remaining alive and functional.

Product	Cat. No	Packing specification	Transporting condition	Preser-vating condition	DMSO	Trehalose	Cell viability after thawing*
ImmuCryo-save	342 343	100 mL 500 mL	2-8°C	2-8°C	Có 5%	No	>90%
ImmuCryo-save OTS TH	344 345	100 mL 500 mL	RT	RT	Không	Yes	>90%

**Cell viability after thawing is evaluated by two methods: counting on a hemocytometer with Trypan Blue and on a flow cytometer with 7-AAD staining solution. Results were normalized with the percentage of dead cells before freezing. The viability of NK cells after thawing is significantly impacted by their condition prior to freezing. This conclusion is drawn from the observation of NK cells proliferated for 15 days using NKCult kit.*

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