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Expression Time and Peak Concentration Dendritic Cells, Perforin, and Granzyme B in Invivo and Invitro Studies: A Systematic Review

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ABSTRACT

Dendritic cells, perforin, and granzyme B are pivotal in immune regulation, yet the timing of their expression and peak concentration remains variably reported across studies. This systematic review aimed to summarize and compare the expression time and peak concentration of dendritic cells, perforin, and granzyme B from in vivo and in vitro studies. A systematic search was conducted in PubMed, ScienceDirect, ResearchGate, and Google Scholar for studies published between 1990 and 2024. Eligible studies included experimental in vitro, in vivo, or clinical investigations reporting temporal expression or peak concentration of DCs, perforin, or granzymes. Data were extracted on study design, methods, and outcomes, and risk of bias was assessed using SYRCLE's and Cochrane tools. From 732 identified records, 11 studies met inclusion criteria. In vitro studies consistently demonstrated that DCs reached optimal antigen-presenting function on day 7, with immature DCs (day 5) showing stronger antigen uptake. Perforin expression was detected early, peaking around days 2–4 across murine $CD8^+$ T cell activation, influenza infection, uterine implantation, and kidney transplant rejection. Granzyme B followed a similar rapid induction, rising at days 2–3 and peaking at days 3–4, sustaining cytotoxic T cell activity. In contrast, granzyme A and C appeared later (days 5–7), providing complementary effector functions. Translational studies demonstrated the biomarker potential of these molecules: perforin mRNA peaked at the onset of acute rejection, while granzyme B expression and PET imaging distinguished responders from non-responders to immunotherapy. Dendritic cell maturation, perforin, and granzymes display distinct yet coordinated temporal dynamics. DCs optimize antigen presentation by day 7, perforin and granzyme B mediate rapid early cytotoxic responses, and granzyme A/C contribute to later effector phases. These findings provide mechanistic insight into immune regulation and support the clinical utility of perforin and granzyme B as biomarkers of immune activation and therapeutic response.

Keywords: *Interdisciplinary collaboration, post-disaster risk communication, UNESCO World Heritage Site*

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1. INTRODUCTION

The innate immune system includes several defense mechanisms used by the body, including various types of immune cells such as granulocytes (neutrophils, eosinophils, basophils), natural killer cells, mast cells, and presenting cells (APCs) such as monocytes, macrophages, and dendritic cells [1]. Meanwhile, the adaptive immune system has two main mechanisms: humoral and adaptive. Humoral immunity is mainly mediated by B cells and antibodies, while cellular immunity is led by T cells [2].

T-cell activation is preceded by antigen presentation by antigen-presenting cells such as macrophages, B cells, and dendritic cells. Dendritic cells recognize pathogens and process antigens before migrating to lymph nodes to present these antigens via MHC. Antigen presentation involves exogenous and endogenous antigens via MHC II and MHC I. Costimulatory signals, mediated by B7 proteins on dendritic cells that interact with CD28 on naive T cells. In addition to functioning to

recognize antigens, process them, and present them to T cells, dendritic cells also play a role in the development of immunological memory that protects the body from repeated bouts of infection [3], [4]..

by cytotoxic T cells (CD8+) and natural killer (NK) cells. This protein destroys target cells, such as virus-infected cells or cancer cells. Perforin forms pores in the target cell membrane, allowing other effector molecules, such as granzymes, to enter the cell. Granzymes, especially granzyme B, then function to induce apoptosis or programmed cell death [4], [5]. This study aims to summarize and analyze data on the expression time and peak concentration of dendritic cells, perforin, and granzyme B from previous in vivo and in vitro studies.

2. METHODOLOGY

The present study was conducted in three stages. The first stage included the review of previous literatures and structures of relevant government agencies in the context of this study. The geographical, cultural, social and other aspects of the area were then observed on site, and finally climatic factors that could affect the situation were well-known. The following subsections describe the methodology and approach in details.

2.1 Data Source and Data Strategy

A systematic and comprehensive literature search was performed using PubMed, ScienceDirect, ResearchGate, and Google Scholar for studies published between 1990 and 2024. The search strategy employed the terms “dendritic cells,” “perforin,” and “granzyme B” both individually and in combination, with Boolean operators (“AND” and “OR”) applied to optimize sensitivity and specificity. In addition to the electronic search, the reference lists of relevant articles were screened manually to identify additional eligible studies not captured in the initial search.

2.2 Inclusion and Exclusion Criteria

Studies were included if they were original research articles published in English and reported data on the expression time or peak concentration of dendritic cells, perforin, or granzyme B. Eligible study designs encompassed experimental in vivo and in vitro studies as well as prospective cohort studies. Articles were excluded if they were reviews, editorials, or conference abstracts, if they lacked sufficient or extractable data, or if they did not address the relevant expression time or peak concentration outcomes.

2.3 Data Extraction and Analysis

Two independent reviewers screened all records by title, abstract, and full text to assess eligibility. Disagreements were resolved through discussion until consensus was reached. From the eligible studies, the following data were extracted: author(s) and year of publication, target molecules investigated, study design and research model, study subjects (human, animal, or primary cells), methods of observation (histology, PCR, flow cytometry, western blot, immunohistochemistry, immunofluorescence, or in situ hybridization), observation period, expression time, and peak concentration. Extracted data were analyzed descriptively and summarized in comparative tables to facilitate interpretation and highlight trends across studies.

2.4Risk of Bias Analysis

We assessed risk of bias separately by study design, using validated tools and a pre-specified workflow. Primary assessment used the SYRCLE risk of bias tool (domain structure mirroring Cochrane but tailored to preclinical experiments). Domains evaluated were: sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers/investigators, random outcome assessment, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias (e.g., unit-of-analysis errors, inappropriate statistical methods, batch effects). Judgement categories were low, high, or unclear risk. For in vitro/ex vivo studies without animals, we applied an a priori adaptation of SYRCLE focusing on: randomization of wells/samples, concealment of allocation across plates/batches, blinding of personnel and outcome assessors (including automated readouts), management of missing data and replication, prespecification of endpoints/timepoints, and selective reporting (protocol/methods vs results consistency) [6].

Two reviewers independently piloted the forms on a subset of studies (calibration), then assessed all included records in duplicate. Disagreements were resolved by discussion; a third reviewer arbitrated if needed. We recorded rationales for each domain judgement. Where information was insufficient, authors’ methods/supplements were scrutinized; if still unclear, domains were marked unclear/no information.

For time-course/cellular assays, the biological replicate (animal or donor) was treated as the analysis unit; technical replicates informed precision only. We noted risks of pseudoreplication, unplanned interim looks, and multiplicity without adjustment under “other bias”. Methods and presentation of the risk-of-bias process followed PRISMA 2020 guidance for systematic reviews [7].

3. FINDINGS FROM THE FIELDWORK

Study Selection

The initial literature search across PubMed, ScienceDirect, ResearchGate, and Google Scholar yielded a total of 732 records. After removal of 176 duplicates, 556 studies remained for title and abstract screening. Of these, 432 articles were excluded for not meeting the eligibility criteria, leaving 124 articles for full-text assessment.

Following full-text review, 113 studies were excluded due to irrelevance to the review objectives (e.g., lacking data on dendritic cells, perforin, or granzyme expression kinetics; insufficient reporting of outcomes; or inappropriate study design). Ultimately, 11 studies fulfilled the inclusion criteria and were incorporated into the qualitative synthesis.

The detailed selection process is presented in the PRISMA flow diagram (Figure 1).

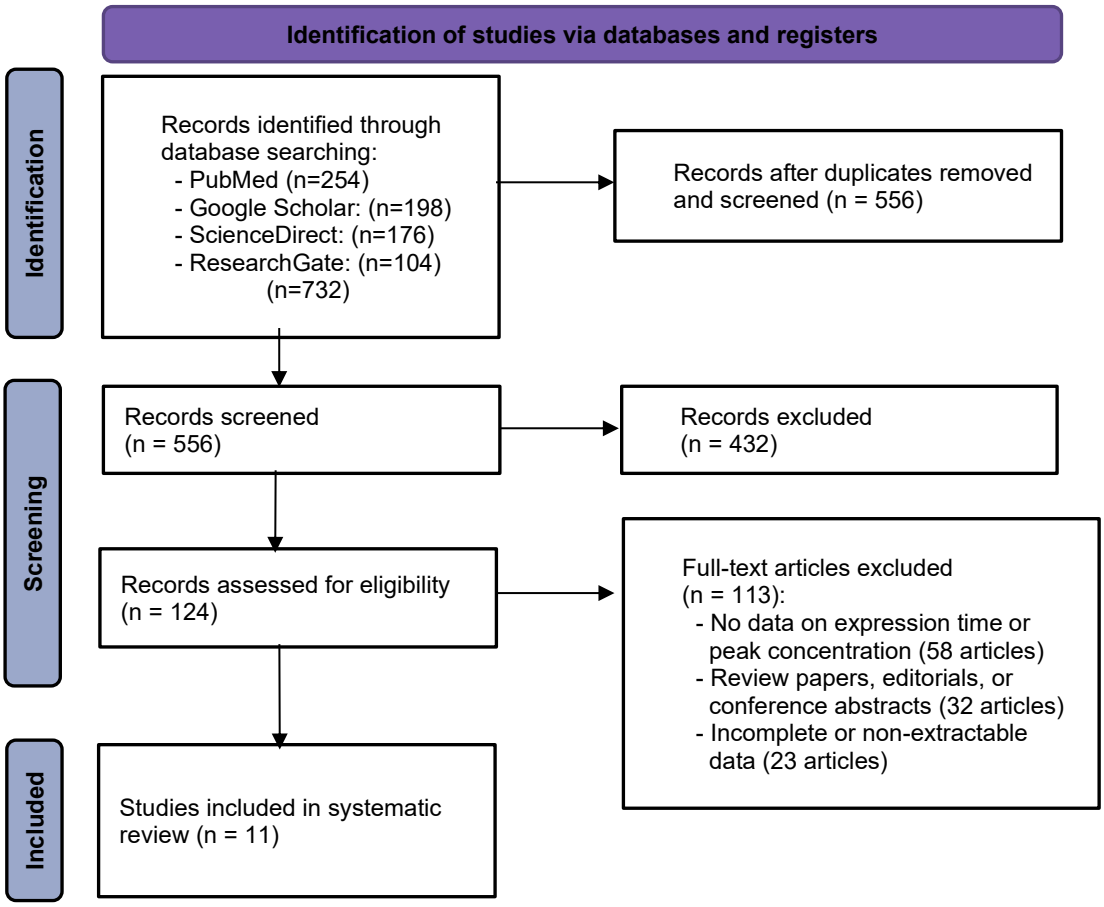

Figure 1: PRISMA Flow Diagram Study Selection Process

Study Characteristics

A total of 11 studies published between 1991 and 2021 were included, comprising six in vitro/ex vivo studies, four in vivo animal studies, and two clinical studies (one translational with both animal and human cohorts). The studies were conducted across multiple countries, including the USA, Japan, Israel, UK, Korea, Australia, and the Netherlands, reflecting diverse research contexts (Table 1).

In vitro and ex vivo investigations primarily focused on dendritic cell maturation and cytotoxic gene regulation. Studies of human monocyte-derived dendritic cells demonstrated that optimal maturation with high expression of MHC-II and co-stimulatory molecules occurred on day 7 of culture, whereas earlier time points (day 5) exhibited stronger antigen uptake capacity. Additional in vitro experiments revealed that IL-2 directly upregulates perforin and granzyme expression in murine CD8+ T cells, while single-cell PCR analyses showed distinct temporal patterns, with granzyme B and IFN-γ induced as early as day 2–3, perforin peaking around day 4, and granzyme A/C expressed later at day 5–7.

Animal studies addressed the kinetics of cytotoxic molecules during immune responses. In murine models of graft rejection, CTLs shifted from a perforin^high to a FasL^high phenotype, while in pregnancy, granulated metrial gland cells expressed high levels of perforin immediately after implantation. During influenza infection, lung-infiltrating CD8+ T cells exhibited

high perforin and granzyme A/B expression, highlighting site-specific effector responses. In cancer immunotherapy models, granzyme B PET imaging successfully distinguished responders from non-responders, suggesting its utility as a predictive biomarker.

Clinical investigations provided translational insights. In kidney transplant recipients, perforin mRNA levels peaked on days 0–1 of acute rejection, indicating potential as an early diagnostic marker. In melanoma patients treated with checkpoint inhibitors, granzyme B expression was significantly higher in responders, consistent with the findings from preclinical PET imaging studies.

Collectively, the included studies highlighted a consistent pattern in which dendritic cells reach functional maturity at day 7, perforin peaks in early phases of immune activation (days 0–4), and granzyme B rises rapidly (days 2–4) with sustained activity in effective cytotoxic responses, while granzyme A and C appear later (days 5–7). These findings underscore the temporally distinct yet complementary roles of dendritic cells, perforin, and granzymes in shaping immune defense and clinical outcomes.

Table 1: Characteristics of Included Studies

A. In vitro / Ex vivo Studies

Author / Year	Country	Model / Population	Design	Methods / Intervention	Outcomes	Key Findings
Grossman et al., 2004 [8]	USA	Human PBMC subsets	Ex vivo	Single-cell analysis of GzmA/B, perforin	Expression frequency	GzmB predominant in CD8+ & NK; GzmA heterogeneously distributed.
Watanabe et al., 2021 [9]	Japan	Human CD56^dim DCs	Ex vivo	Phenotypic & transcriptomic profiling	Cytotoxic markers	Identified DC subset with high GZMB, perforin, PI-9.
Woodhead et al., 1998 [10]	UK	Human monocyte-derived DCs	In vitro	GM-CSF + IL-4 culture (7 days)	Maturation markers	Day 7 DCs: ↑MHC-II, CD80/86 → mature phenotype.
Ishri et al., 2004 [11]	Japan	Human DC culture (d5 vs d7)	In vitro	Monocyte culture	Antigen uptake vs presentation	DC d5 → strong uptake; DC d7 → mature costimulatory phenotype.
Janas et al., 2005 [12]	USA	Murine CD8+ T cells	In vitro	IL-2 stimulation	Perforin & granzymes expression	IL-2 directly induces perforin & granzymes, independent of survival.
Kelso et al., 2002 [13]	Australia	Murine naïve CD8+ T cells	In vitro	Single-cell PCR (day 2–7)	Perforin, GzmA–C, IFN-γ	GzmB + IFN-γ day 2–3; perforin day 4; GzmA/C later (day 5–7).

B. In vivo Animal Studies

Author / Year	Country	Model / Population	Design	Methods / Intervention	Outcomes	Key Findings
Meiraz et al., 2009 [14]	Israel	Murine skin allograft	In vivo	CTL kinetics, flow cytometry	Perforin, FasL, Gzm	CTLs shift from perforin^high to FasL^high during rejection.
Zheng et al., 1991 [15]	USA	Murine uterus (pregnancy)	In vivo	IHC of GMG cells	Perforin	High perforin post-implantation, regulated by maternal decidual factors.
Johnson et al., 2003 [16]	Australia / Netherlands	Murine influenza	In vivo	Single-cell RT-PCR	Perforin, GzmA–C, IFN-γ	Lung effector CD8+ T cells ↑perforin + GzmA/B; MLN cells lower.

C. Translational (Animal + Human)

Author / Year	Country	Model / Population	Design	Key Methods	Outcomes	Outcomes / Key Findings
Larimer et al. 2017 [17]	USA	Murine cancer models + human melanoma	Preclinical + human translational	Granzyme-B PET + tissue analysis	Imaging biomarker	GzmB imaging/expressions distinguish immunotherapy responders vs non-responders.

D. Clinical Studies

Author / Year	Country	Model / Population	Design	Methods / Intervention	Outcomes	Key Findings
Shin et al., 2005 [18]	Korea	Kidney transplant patients (n=15)	Prospective observational	Serial PCR of PBLs	Perforin, GzmB, FasL	Perforin mRNA peaked at day 0–1 of acute rejection, potential biomarker.

Table 2: Risk of Bias Assessment

No	Study	Design	Randomization / Allocation	Blinding	Incomplete Data	Selective Reporting	Overall Risk
1	Grossman et al., 2004	Ex vivo human cells	✗ (not randomized)	✗ (lab-based, no blinding)	Low	Low	Moderate
2	Watanabe et al., 2021	Human ex vivo DC	✗ (observational)	✗	Low	Low	Moderate
3	Meiraz et al., 2009	Murine allograft model	Not clearly reported	✗	Low	Low	Moderate
4	Woodhead et al., 1998	Human in vitro DC culture	✗	✗	Low	Low	Moderate
5	Ishri et al., 2004	Human monocyte-derived DC culture	✗	✗	Low	Low	Moderate
6	Janas et al., 2005	Murine CD8+ T cell culture	✗	✗	Low	Low	Moderate
7	Zheng et al., 1991	Murine pregnancy model	Randomization not described	✗	Low	Low	Moderate
8	Johnson et al., 2003	Murine influenza infection	Randomization not described	✗	Low	Low	Moderate
9	Larimer et al., 2017	Murine + human immunotherapy	Animal part: unclear randomization; Human biopsy: observational	✗	Low	Low	Moderate
10	Shin et al., 2005	Human prospective (transplant patients)	✗ (observational)	✗	Low	Low	Moderate
11	Kelso et al., 2002	Murine CD8+ T cell culture	✗	✗	Low	Low	Moderate

Risk of Bias Analysis

Risk of bias was assessed using the SYRCLE's risk of bias tool for animal and in vitro experimental studies and the Cochrane risk of bias framework for clinical investigations. Overall, most of the included studies demonstrated moderate risk of bias, largely due to methodological limitations inherent to laboratory and preclinical designs (Table 2).

Across the in vitro and animal studies, domains such as random sequence generation, allocation concealment, and blinding were generally not reported, as these studies were primarily mechanistic laboratory experiments rather than controlled randomized trials. Nevertheless, the majority of studies provided complete outcome data and showed low risk of selective reporting, as the primary endpoints (gene or protein expression of dendritic cells, perforin, or granzymes) were clearly defined and consistently measured.

For the animal models (e.g., graft rejection, influenza infection, pregnancy, and immunotherapy), none of the studies explicitly described randomization procedures or blinding of outcome assessment. This lack of methodological detail places them in the unclear-to-moderate risk category. However, the experimental designs were internally consistent, and laboratory assays (such as RT-PCR, flow cytometry, and immunohistochemistry) were standardized, which reduces the likelihood of reporting bias.

In the clinical studies, both Shin et al. (2005) on kidney transplantation and Larimer et al. (2017) on melanoma patients used observational or translational designs. Neither study reported randomization or blinding, but both demonstrated low attrition bias with complete follow-up and robust outcome reporting. As such, the overall risk of bias for clinical studies was rated as moderate, reflecting methodological constraints but not major threats to validity.

In summary, the risk of bias across the included studies was predominantly moderate, driven by insufficient reporting of randomization and blinding. Despite these limitations, outcome measures were direct, standardized, and consistently reported, lending reasonable confidence to the synthesized findings.

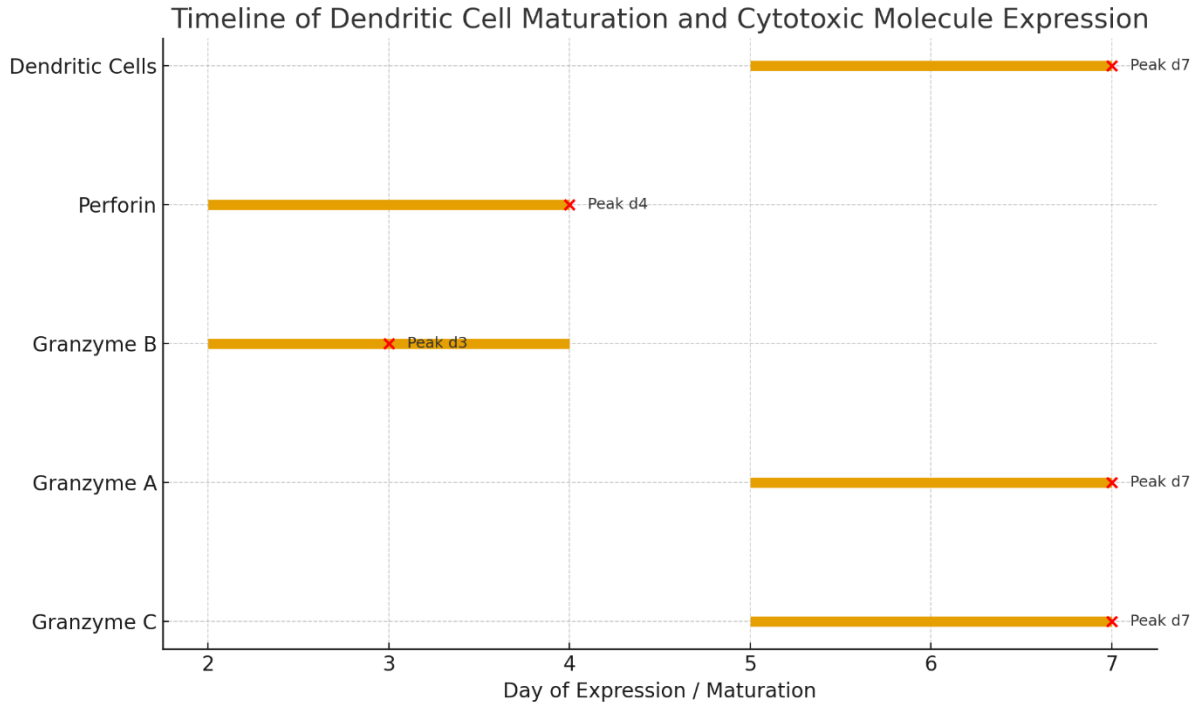

Figure 2: Timeline of Dendritic Cell Maturation and Cytotoxic Molecule Expression

Figure 2 illustrates the temporal kinetics of dendritic cell maturation and cytotoxic molecule expression. Immature dendritic cells begin differentiating around day 5, achieving full maturation with high antigen-presenting capacity by day 7. Perforin expression emerges rapidly during immune activation, detectable by day 2 and peaking around day 4, in line with its role as an early effector of cytotoxicity. Granzyme B shows a similar early induction, rising sharply at days 2–3 and reaching peak levels by days 3–4, sustaining cytotoxic T cell activity throughout the effector phase. In contrast, granzyme A and granzyme C appear later, typically from day 5 onward, with peak expression around day 7.

This sequential pattern highlights the complementary timing of immune effectors, whereby dendritic cells first achieve maturation to optimize antigen presentation, followed by rapid perforin and granzyme B responses for immediate cytotoxicity, and later induction of granzyme A and C to sustain and diversify immune defense mechanisms.

4. DISCUSSION

4.1 Dendritic Cell Expression

Two studies investigated dendritic cell (DC) differentiation and function in vitro. Human monocyte-derived DCs demonstrated a clear temporal pattern, with immature DCs on day 5 showing high antigen uptake capacity but limited costimulatory potential, whereas by day 7, DCs had acquired a fully mature phenotype characterized by upregulated MHC-II and costimulatory molecules (CD80, CD86) [10], [11]. These findings indicate that day 7 represents the optimal time point for antigen presentation and T-cell activation.Regarding functionality and marker expression, dendritic cells isolated on day 5 (d5DC) tend to be immature compared to day 7. On day 7 (d7DC), the expression of markers CD80, CD83, CD86, and MHC II was at a higher level, so the function of dendritic cells as antigen-presenting cells (APC) was stronger at that time. However, on day 5, phagocytosis and antigen processing activities tend to be higher.7 This is in line with research conducted by Ishri et al. (2004); dendritic cells generated on day 5 (d5DC) had a less mature phenotype than dendritic cells generated on day 7 (d7DC). d7DC expressed phenotypic markers that signify maturation such as CD11c, CD80, CD83, CD86, and MHC class II in higher levels. However, d5DCs have better antigen processing ability characterized by higher macropinocyte activity, phagocytosis, and proteolytic enzyme activities such as cathepsins C and G in d5DCs compared to d7DCs [11]. Thus, dendritic cells isolated on day 7 are better inducers of immune responses due to their stronger T lymphocyte stimulation ability.

The transition from monocytes to dendritic cells occurred on day 2. Characterized by the expression of CD1a and CD86, antigen-presenting markers increased sharply in the first 2-3 days of culture, then reached a plateau until day 7. Initial activation of monocyte cells occurred within the first 24 hours and was characterized by increased expression of activation markers such as HLA-DR, CD13, CD14, and CD98. After 24 hours, a clear distinction between large and small cell populations based on size and density indicated early morphological changes. The expression of CD14, a monocyte marker, decreased dramatically after 3 days of culture and stayed at a baseline level in the following 3 days, indicating a phenotype transition towards non-monocytic. Increased expression of antigen presentation markers such as HLA-DR, HLA-DQ, CD1a, and CD86 was only seen after a few days, indicating continued cell maturity [10].

Dendritic cells (DCs) are one of the antigen-presenting cells (APCs) capable of capable of activating thousands of naïve T cells through effective peptide presentation on MHC molecules [19]. Beyond antigen uptake and presentation, they regulate selective immune responses, promote tolerance by inducing regulatory T cells, and act as key immune modulators [20]. Dendritic cells express MHC class II molecules quickly and effectively and can present peptides more than 100 times that of other APCs. One dendritic cell can activate 3000 naive T cells [19].

DC differentiation progresses from precursor to immature and mature stages, originating from myeloid or lymphoid progenitors. Myeloid-derived DCs include monocyte-derived DCs (moDCs), peripheral blood DCs, Langerhans cells, and mesenchymal DCs (CD1a, CD11c, CD14, CD33), while lymphoid-derived pro-DCs express CD123 and BDCA-2 [19], [21].

The involvement of dendritic cells extends beyond mere antigen capture and presentation, as they tailor selective immune responses against different classes of pathogens. Moreover, complex branch structures, known as dendritic arbors, allow dendritic cells to receive signals from various cells, underscoring the role of dendritic cells as key regulators of immune responses [22]. Also, dendritic cells contribute to immune tolerance by inducing regulatory T cells that prevent the immune system from attacking the body's tissues [23].

Functionally, DCs are classified into [21]:

1. Conventional DCs (cDC1 and cDC2) – cDC1 (XCR1+, CD103+) promote CD8+ T cell and Th1/Th17 responses; cDC2 (CD11b+) are linked to Th2/Th17 activation.
2. Monocyte-derived DCs (moDCs) – arise during inflammation from Ly6C^hi monocytes via CCR2, supporting Th1 responses.
3. Plasmacytoid DCs (pDCs) – major producers of type I interferon, critical for antiviral immunity.
4. Langerhans cells (LCs) – epidermal DCs with dual macrophage/DC traits; upon activation, they migrate to lymph nodes and can either stimulate or regulate T cell responses.

4.2 Perforin Expression Dynamics

Perforin expression was consistently observed as an early event across multiple models. In murine CD8+ T cell activation, perforin mRNA was induced by day 2 and peaked by day 4 [13]. In lung CD8+ T cells responding to influenza infection, perforin was highly expressed at the site of infection compared with draining lymph nodes [16]. In reproductive immunology, uterine granulated metrial gland (GMG) cells displayed strong perforin expression immediately after implantation [15]. Clinically, kidney transplant recipients undergoing acute rejection demonstrated a surge in perforin mRNA at days 0–1 of the rejection crisis [18]. Collectively, these data highlight perforin as a rapid-response effector

molecule in diverse immunological contexts.

Perforin is a protein utilized by cytotoxic T cells (CTL) and natural killer cells (NK). Perforins play an essential role in cell-mediated immunity by penetrating the membranes of target cells for easy eradication [21]. Perforins are released onto the membranes of identified target cells infected by viruses or cancer. These perforin molecules polymerize to form ring-shaped structures and form pores in the membrane. The pores serve as gateways for granzymes, additional molecules released by CTLs and NK cells to enter the target cell and trigger apoptosis [5], [24].

The pores formed by perforin are large enough to allow passive diffusion of granzyme into target cells, estimated to be 8 to 20 nm in diameter. Perforin forms the membrane pore through oligomerization of the MACPF domain [25]. Regulation of perforin activity is controlled to ensure its specificity to the right target by the influence of specific factors on the target cell surface. Perforin deficiency can lead to an increased risk of infection and weakened immunity to some cancers. In autoimmune conditions, perforin can damage healthy tissue due to impaired regulation [5], [24].

The activity of perforin depends on calcium ions (Ca^{2+}), essential for binding to cholesterol and phospholipids in the target cell membrane. Oligomerization of perforin molecules into ring-shaped structures is also dependent on calcium concentration. Once formed, these oligomers enter the target cell membrane, disrupting the stability of its lipid bilayer and triggering the formation of pores. The size and stability of these pores are modulated by factors such as the number of perforin molecules involved and the composition of the target cell membrane [5].

4.3 Granzyme Expression Patterns

Granzyme expression showed distinct kinetics depending on subtype. Granzyme B was consistently the earliest and most robust, with expression detectable as early as day 2–3 and peaking by day 3–4 in activated CD8+ T cells [13]. In influenza-infected mice, granzyme B was abundant in lung-infiltrating CD8+ T cells [16], and imaging studies confirmed its role as a biomarker of cytotoxic activity [17]. In contrast, Granzyme A and C exhibited delayed kinetics, appearing at days 5–7 [13]. Single-cell PCR revealed substantial heterogeneity, with individual T cells expressing diverse combinations of perforin and granzymes rather than uniform profiles [13].

According to observations made by Meiraz et al. on CD8+ T cells isolated from the peritoneal cavity of mice after intraperitoneal transplantation of allogeneic tumor cells, perforin protein was very high on day 8 and decreased dramatically on day 11 [14]. Most CD8+ T cells on day 8 expressed perforin protein, while on day 11, only about 11% of CD8+ T cells expressed perforin. If the antigen continues, perforin expression on CD8+ cells remains high until day 14. This suggests that the decrease in perforin expression is related to the disappearance of the antigen [14].

In patients who experience acute rejection of a kidney transplant, perforin gene expression in peripheral blood lymphocytes significantly increases, especially on day 0 or 1, from the onset of rejection. The peak concentration of perforin expression occurred on days 0 and 1 since the onset of the rise in serum creatinine and remained elevated above the standard threshold until the end of the rejection episode, usually on days 6 to 7. Meanwhile, there was no difference in granzyme B expression between patients who experienced acute rejection and controls, indicating that granzyme B is still expressed in the peripheral blood without the influence of the rejection episode [18].

Granzyme B expression peaks on days 3 to 4 by activating CD4+ T cells via the T cell receptor (CD3) or costimulatory coreceptor (CD46) [15]. According to Larimer et al. (2017), extracellular granzyme B has a biological half-life of 14 days. Western blot analysis found that granzyme B expression in immunotherapy-treated tumors was significantly increased on days 12 and 14 compared to untreated tumors. Based on immunofluorescence images, the expression pattern of granzyme B was different between treated and untreated tumors, with treated tumors showing more extensive and more intense areas of granzyme B expression not always localized with cytotoxic T cells. This indicates the release of granzyme B into the extracellular space after T cell activation [16].

In CD8+ T cells from mouse lymph nodes activated with IL-2, granzyme B peaked on day 3, while perforin expression increased 10-fold on day 4 along the IL-2 concentration gradient.Granzyme B expression increased up to 10,000-fold along the IL-2 concentration gradient [14]. According to Kelso (2002), several factors potentially affect the peak time of perforin and granzyme expression during CD8+ Tcell activation, including:[9]

1. Different genetic regulation for each gene.
2. The need for additional signals to induce gene expression. Granzyme B may only require a T cell receptor (TCR) activation signal as the primary inductor.
3. The process of cell differentiation. Cells take time to gradually express various genes as they develop and differentiate.
4. Genetic location. Granzyme B is located close together on the chromosome, allowing for coregulatory influences.

Cell culture conditions. Cytokine composition and epithelial stimulation under in vitro culture conditions can affect gene expression kinetics

Granzymes are a family of serine protease enzymes found in the cytotoxic granules of CTLs and NK cells. These molecules play an important role in the immune system against various threats, including viral infections, cancer, and intercellular pathogens [1], [26]. Granzymes consist of five known types in humans (A, B, H, K, and M), each with slightly different functions and directed towards a specific substrate within the target cell [27].

Granzyme is retained in an inactive form during its formation in the endoplasmic reticulum (RE) for subsequent glycosylation. Along the RE and Golgi, oligosaccharide groups aid in targeting lysosomes via mannose-6-phosphate receptors. Katespsin C releases propeptides to activate proteolytic activity. The granzyme then binds to the cerglycine and is deposited into granules in the lumen [23].

Granzyme B is one of the most studied and is an apoptotic inductor that directly cleaves and activates caspases while also affecting other cellular proteins involved in DNA repair and cell survival pathways [5].

Granzymes are stored inside the cytotoxic granule along with perforin. CTLs or NK cells release both granzymes and perforin upon target cell recognition. Perforin forms pores in the target cell membrane, allowing the granzyme to enter the cytoplasm and carry out its effects. Once inside the target cell, granzymes can activate various pathways, leading to cell death. They can directly cut important cellular proteins, activate caspases (enzymes responsible for cell breakdown), or disrupt the integrity of the cellular membrane, and end up in cell apoptosis [28].

4.4Detection of Dendritic Cells, Perforin, and Granzyme B in Cell Analysis

Immunofluorescence techniques allow visualization of proteins in cells, while flow cytometry is used to measure and analyze the expression of these proteins in larger cell populations. Both methods have been successfully used to detect perforin in cytotoxic T lymphocytes in mice [29].

Flow cytometry can also detect granzymes on the cell surface and measure cytokine production by CD8+ T cells after stimulation. This technique allows for highly detailed and quantitative cellular analysis. Polychromatic flow cytometry has been used to detect perforin and granzyme expression in human CD8+ cells [30].

In immunohistochemistry, specific monoclonal antibodies are used against perforin and granzyme to label cells in tissue samples. Immunohistochemistry allows visualization of the distribution of these proteins in tissues [31].

Dendritic cells can be detected using specific antibodies such as CD11c, HLA- DR,and other markers. Immunohistochemical techniques allow visualization of dendritic cells in tissues. 32 In a study conducted by Orgad et al., a subpopulation of dendritic cells that produce perforin and granzyme A, also known as "perf-DCs", was detected by immunohistochemistry and flow cytometry [32]. Flow cytometry techniques can also detect and quantify the expression of dendritic cell surface markers on cells isolated from inflammatory tissues. In research, this method can analyze dendritic cells' number and activation status in blood, synovial fluid, or other inflammatory tissues. For example, a study by Canavan et al. (2016) used flow cytometry to examine the immune phenotype of dendritic cells in the synovial fluid of patients with rheumatoid arthritis [33]. In vivo confocal microscopy also allows direct observation of dendritic cells in living tissue, such as cornea or skin [34].

The timing of the appearance and disappearance of perforin, granzyme, and dendritic cells varies depending on the case and phase of the immune response. In HIV infection, perforin and granzyme A can be detected soon after the acute phase of infection [35]. In mice infected with influenza virus, perforin and granzyme A and B expression can be detected in CD8+ T cells in lung tissue after isolation from infected mice.8 In cases of acute rejection in kidney transplantation, increased expression of perforin and granzyme B is found about 3 days before the appearance of clinical symptoms [18].

After the resolution of inflammation or infection, the expression of perforin and granzyme tends to decrease as the cytotoxic activity of T and NK cells decreases. For example, in cases of transplant rejection successfully resolved with immunosuppressant therapy, the expression of perforin and granzyme significantly decreases after several weeks of effective treatment [36]. These molecules will disappear with the control of excessive immune response, such as in immunosuppressant therapy with cyclosporine, which can suppress the expression of perforin and granzyme [37].

4.5The Role of Dendritic Cells, Perforin, and Granzyme B in Wound Healing

Dendritic cells migrate along the epithelial layer to close wounds at the inflammation stage. In a study by Stewart et al.(2012), dendritic cells recruit inflammatory cells such as neutrophils. They can influence the expression of chemokines such as CXCL10 and cytokines such as TSLP on migrating epithelial cells. Both of these mediators are important for recruiting other inflammatory cells and initiating the inflammatory response [23].

Perforin plays a role, especially in the early stages of the hemostasis process and inflammation during the wound healing mechanism, by stimulating the membrane response to clear the damage-causing agent through the apoptotic mechanism of granzyme [36]. The pore formed by perforin allows Ca2+ flux into the cytoplasm and can cause plasma membrane damage. Damage triggered by this Ca2+ influx will provoke a membrane healing response through fusion of intracellular vesicles (lysosomes & endosomes) [38].

O'Neill et al. (2020) also found that perforin-2 (macrophage-expressed gene 1 or MPEG1) plays a role in fighting intracellular bacterial infection during the inflammatory stage of wound healing [39]. Perforin-2 is an antibacterial effector protein expressed in the epidermis and increases after wounding [40].

Granzyme B and dendritic cells are found in the remodeling phase. Granzyme B has strong extracellular matrix remodeling activity. Granzyme B can cleave vitronectin, fibronectin, and laminin, all of which are important in the structure and function of the extracellular matrix. This suggests that granzyme B not only plays a role in apoptosis but also in tissue modification during the remodeling phase [41], [42].

4.6Role of Dendritic Cells, Perforin, and Granzyme B in Pathological Processes

Perforin expression on CD8+ T cells is prognostically significant for predicting the severity of herpes virus reactivation after bone marrow transplantation. There was an increased percentage of perforin expression on CD8+ T cells of patients with viral reactivation after transplantation compared to patients without viral reactivation. The percentage of perforin expression was higher in patients with high viral load, indicating that perforin correlated with the severity of viral reactivation [43].

Based on research conducted by Pastar et al., perforin-2 expression in gamma delta T cells, basal keratinocytes, and papillary fibroblasts in human skin can be induced by *Staphylococcus epidermidis* which is a commensal microbe in the skin. *Methicillin-resistant Staphylococcus aureus (MRSA) is known to suppress perforin-2 expression as a mechanism to evade the skin's* immune system. S. epidermidis as a skin commensal can modulate the human skin's innate immune response by stimulating the activation of gamma delta T cells and inducing perforin- 2 expression to protect against pathogenic infections such as MRSA [44].

Perforin and granzyme B, in their physiological roles, function to recognize and eradicate foreign cells. However, in tumor types that can avoid immune surveillance, such as spleen tumor models, melanoma, and acute myeloblastic leukemia in mice, the role of perforin and granzyme B can be avoided. According to Cao et al. (2007), regulatory T cells (Treg) can suppress immune responses against tumors. Tumor cells utilize Tregs to evade recognition and attack by the immune system, while Tregs use perforin and granzyme B to kill cytotoxic NK and CD8+ cells [45]**.**

4.7Integration of Cytotoxic Profiles in Allograft and Tumor Models

In murine allograft rejection, cytotoxic T lymphocytes transitioned from a perforin^high to a FasL^high phenotype, suggesting a dynamic shift in effector mechanisms during the course of rejection [illegible]. In tumor models, a distinct subset of CD56^dim dendritic cells with high GZMB, perforin, and PI-9 expression was identified [illegible], supporting their potential tumoricidal role. Preclinical PET imaging of granzyme B further demonstrated the capacity to distinguish responders from non-responders to checkpoint blockade therapy [illegible].

4.8Clinical Relevance and Biomarker Potential

Two studies highlighted the translational relevance of perforin and granzyme B as biomarkers. In kidney transplantation, perforin expression in peripheral blood lymphocytes provided an early and noninvasive marker of acute rejection [illegible]. In melanoma patients treated with immunotherapy, granzyme B expression detected by PET imaging and tissue analysis correlated strongly with clinical response [illegible]. These findings underscore the potential of cytotoxic effector molecules not only as mechanistic insights but also as clinical biomarkers.

4.9Summary of Findings

Across 11 studies, a consistent temporal pattern was observed: dendritic cells achieve functional maturity at day 7, perforin expression peaks early (day 0–4), granzyme B rises rapidly (day 2–4) and sustains cytotoxic activity, while granzyme A and C appear later (day 5–7). These temporally distinct expression patterns were evident across in vitro, in vivo, and clinical settings, highlighting their complementary roles in immune activation, cytotoxicity, and regulation.

5. CONCLUSION AND WAY FORWARD

This systematic review highlights a consistent temporal pattern in dendritic cell maturation and cytotoxic effector molecule expression. Dendritic cells achieve optimal antigen-presenting capacity at day 7, coinciding with the initiation of effective T cell activation. Perforin and granzyme B are rapidly induced during the early effector phase, peaking between days 2–4, and play a critical role in immediate cytotoxicity. In contrast, granzyme A and C appear later (days 5–7), providing sustained and complementary functions. Evidence from clinical studies further supports the translational relevance of perforin and granzyme B as early biomarkers of immune activation, including transplant rejection and responsiveness to immunotherapy. Taken together, these findings underscore the temporally distinct yet coordinated roles of dendritic cells, perforin, and granzymes in shaping immune defense and regulating clinical outcomes

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