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# Immune Niches within Tissues: Histological Insights into Localized Immune Regulation: A review article

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**Abstract :**

The immune system operates not only on a systemic level but also through finely tuned localized responses within specific tissue environments. These specialized microenvironments, known as immune niches, play a pivotal role in regulating immune cell behavior, maintaining homeostasis, and responding to injury or infection. Immune niches are formed by a complex interplay of immune cells, stromal cells, vascular structures, and extracellular matrix components, creating distinct spatial and functional compartments tailored to the needs of each tissue. Histological analysis using advanced imaging techniques, such as immunohistochemistry and multiplex immunofluorescence, has greatly enhanced our understanding of the architecture and cellular composition of these niches. For example, in the gut and skin, distinct immune niches support tolerance to commensal microbes while remaining responsive to pathogens. In contrast, in chronic diseases such as cancer or autoimmune disorders, the disruption or reprogramming of these niches can drive disease progression by promoting immune evasion or sustained inflammation. Histological studies allow for the visualization of cellular localization, interaction, and activation states within the native tissue context, providing essential insight into the dynamic regulation of local immunity. Understanding immune niches from a histological perspective opens new avenues for tissue-specific immunotherapies and precision medicine. By targeting the unique cellular and molecular features of these localized environments, it is possible to design more effective and less toxic therapeutic strategies. This review aims to explore and synthesize current histological evidence on the organization and function of immune niches within various tissues. It seeks to highlight how localized microenvironments regulate immune cell behavior, contribute to tissue homeostasis, and influence immune responses in both physiological and pathological contexts.

**Key word:** Immune niches, Tissue-specific immunity, Stromal cells, Histological analysis, Localized immune regulation.

# المنافذ المناعية داخل الأنسجة: رؤى نسيجية حول التنظيم المناعي الموضعي/ مراجعة مقال

مروة خليل إبراهيم ، نور محمد جعفر حمودي ، رنا علاء العامري
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**مستخلص:**

لا يعمل الجهاز المناعي على المستوى الجهازي فحسب، بل أيضًا من خلال استجابات موضعية دقيقة ضمن بيئات نسيجية محددة. تلعب هذه البيئات الدقيقة المتخصصة، المعروفة باسم المنافذ المناعية، دورًا محوريًا في تنظيم سلوك الخلايا المناعية، والحفاظ على التوازن الداخلي، والاستجابة للإصابة أو العدوى. تتشكل المنافذ المناعية من خلال تفاعل معقد بين الخلايا المناعية، والخلايا السدوية، والتراكيب الوعائية، ومكونات المصفوفة الخارج خلوية، مما يخلق مساحات مكانية ووظيفية مميزة مصممة خصيصًا لتلبية احتياجات كل نسيج. وقد عزز التحليل النسيجي باستخدام تقنيات التصوير المتقدمة، مثل الكيمياء المناعية النسجية والتألق المناعي المتعدد، فهمنا بشكل كبير لتركيب هذه المنافذ وتكوينها الخلوي. على سبيل المثال، في الأمعاء والجلد، تدعم منافذ مناعية مميزة تحمل الميكروبات المتعايشة مع الحفاظ على استجابتها لمسببات الأمراض. في المقابل، في الأمراض المزمنة كالسرطان أو اضطرابات المناعة الذاتية، قد يؤدي تعطيل أو إعادة برمجة هذه المنافذ إلى تفاقم المرض من خلال تعزيز التهرب المناعي أو الالتهاب المستمر. تتيح الدراسات النسيجية تصور مواقع الخلايا وتفاعلها وحالات تنشيطها ضمن سياق الأنسجة الأصلية، مما يوفر فهمًا أساسيًا للتنظيم الديناميكي للمناعة الموقعية. إن فهم المنافذ المناعية من منظور نسيجي يفتح آفاقًا جديدة للعلاجات المناعية المتخصصة بالأنسجة والطب الدقيق. ومن خلال استهداف السمات الخلوية والجزيئية الفريدة لهذه البيئات الموضعية، يُمكن تصميم استراتيجيات علاجية أكثر فعالية وأقل سمية. تهدف هذه المراجعة إلى استكشاف وتجميع الأدلة النسيجية الحالية حول تنظيم ووظيفة المنافذ المناعية داخل الأنسجة المختلفة. وتسعى إلى توضيح كيف تنظم البيئات الدقيقة المحلية سلوك خلايا المناعة، وتسهم في الحفاظ على توازن الأنسجة، وتؤثر على الاستجابات المناعية في كل من السياقات الفسيولوجية والمرضية.

**الكلمات المفتاحية:** المنافذ المناعية، المناعة المتخصصة بالأنسجة، الخلايا السدوية، التحليل النسيجي، التنظيم المناعي الموضعي.

## 1. Introduction

Immune niches are crucial for maintaining homeostasis and play a pivotal role in organizing and maintaining a cell type's function. High-throughput imaging technologies reveal similar fixed structures and dynamic properties in tissues. [1].

Immune niches are specialized areas with antigen-presenting cells, providing a specialized environment for lymphocytes to propagate and interact, prompting the search for new immune organs [2].

Immune niches, including organized tissues like lymph nodes and spleen, are crucial for regulating immune responses. Recent advances in histological techniques offer new research opportunities for understanding these niches in human tissues [3](Figure 1).

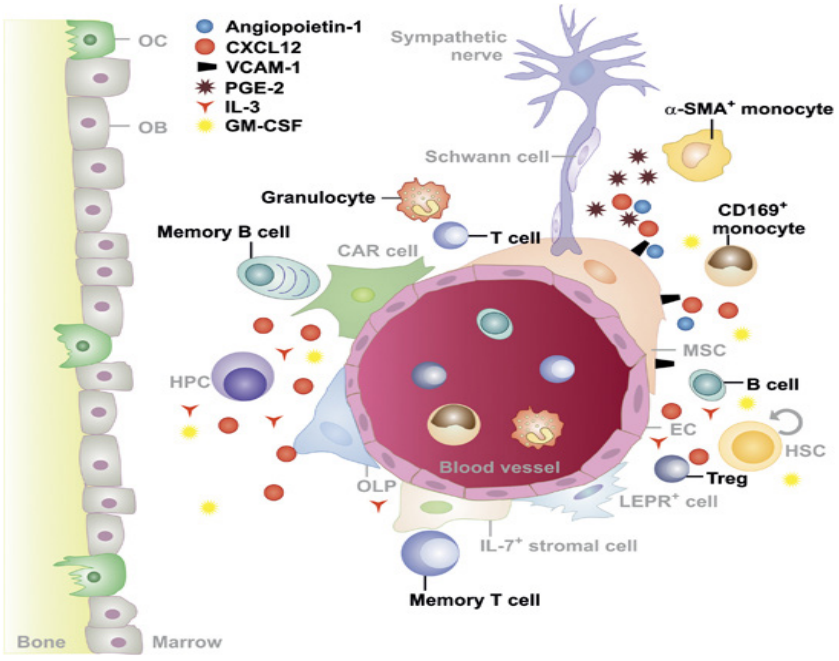


Figure (1): The immune niche [4]

Immune organ formation is influenced by environmental factors, gene expression, and signaling pathways during embryonic development. Niches support priming and differentiation of T and B cells. Search for immune niches involves activating specific T cells and germinal center-like structures [5].

## 2. Techniques used in immunohistology

Advances in immunological methods have improved understanding of tissue-specific immune responses, leading to the definition of immune niches. However, most knowledge comes from mouse models or non-human primates. This review details existing histological techniques, including whole-mount immunohistochemistry, planar immunohistochemistry, scRNAseq-based disaggregation methods, and mass spectrometry and fluorescence multiplexing analysis, aiming to stimulate further research on tissue immune organization and functions [6]. High-throughput methods are needed to study immune compartments in tissues and immune-niches outside lymphoid organs. Combining PSA-FISH with whole-slide image analysis can provide better understanding of cellular organization in lumen-containing tissues and tissues with abundant cellular components [7, 8]. The Flexiomics platform extracts tissue sections from 1 $cm^3$ samples for fluorescence or mass-spectroscopy profiling, with recent software enhancing histological IHC staining analysis, increasing sample analysis for inter-group differences [9].

### 2.1. Tissue Preparation Methods

Understanding immune regulation in tissue and organ-specific ways requires understanding techniques. Two main procedures involve tissue preparation and histological processing of paraffin-embedded tissue specimens, and a new 3D tissue preparation for advanced tissue-clearing and imaging approaches [10].

Tissues can be easily prepared for histological investigation using archival specimens. Immunohistochemical or immunofluorescent labels and satellite imaging or sections allow high-multiplex examination of whole tissues, slices, and sections for immuno-oncology and tissue immunology [11]. However, prospective studies require more laborious pre-treatment of fresh tissues, such as simple immersion fixation [12]. Transgenic mice were re-anesthetized, excised, and fixed with 4% paraformaldehyde. They were then washed and embedded in low melt agarose-embedding medium [13].

### 2.2. Staining Techniques

Tissue architecture influences cellu-

lar interactions, regulating physiological and pathophysiological processes. Histological methods, including classical H&E staining, immunohistochemistry (IHC), and hybridization, provide insights into immune activation and regulation within tissues before multiplex and multiparameter analyses [14].

Histological tissue assessment involves fixation, dehydration, and embedding in paraffin. Thin sections are deparaffinized, rehydrated, and stained. H&E staining uses haematoxylin solution, eosin solution is applied, and sections are mounted for observation. Immunohistochemistry (IHC) uses hydrogen peroxide and secondary antibody serum [15]. Secondary antibodies detect primary antibodies by conjugating them to enzymes for visible signal detection or fluorochromes for flexible observation, preventing non-specific staining or autofluorescence [16].

Multiplex IHC staining protocols are used on FFPE tissue sections, including simultaneous multiplication and sequential staining. With expanders and polymeric systems, fluorescent-multiplex IHC staining on 30-80 antigens is feasible. Fluorescence in situ hybridization can detect RNA species in fixed tissue sections [17]. Recent poly (adenosine diphosphate-ribose)-specific reagents improve staining in IHC, enabling spatially distinct immune cell populations. However, multiplex imaging requires complex protocols, clarity treatment, and specialized systems [18].

### 2.3. Microscopic Method

Immunological inductive tissues, including secondary lymphoid organs, have unique microenvironments facilitating immune responses to pathogens and immunogenic entities. These microenvironments consist of specialized immune and stromal cell populations, soluble factors, chemokines, and receptors [19]. Imaging platforms that visualize fluorescently labeled cells offer crucial information about molecular and cellular interactions, enabling the development of an immune response to pathogens [20]. Combining imaging platforms with other technologies can address complex issues about immune, endothelial, and stromal populations' *in vivo* behavior and regulation [21]. Immune cells interact with non-immune cells in specific tissues to regulate their fate and function. They migrate from blood flow to organ pa-

renchyma, requiring interactions with endothelial cells. Transgenic mice expressing GFP were used for intravital imaging of lymphocyte migration in lymph nodes [22, 23].

## 3. Types of Immune Niches

Immune-regulatory microenvironments within tissues support immune cell maturation and activation. Classifying immune niches based on immune cell dynamic and cellular composition distinguishes different types [24]. The immune-cell dynamic can be categorized into three types: local cell accumulation, resident cell activation, and local-free access. Cellular composition can be divided into four types: multiple-accessible, specialized, parenchymal, and immature [25].

### 3.1 Antigen-draining niches (ADNs)

Immune cells migrate to draining lymph nodes to localize responses, creating AdNs. This pattern was first observed in CD4 T+ cells responding to ovalbumin. Steady-state and LPS-injected lymph nodes are also classified as AdNs, with DCs and T+ cells contributing to additional accumulation [26].

### 3.2 Local immune-cell-activation niches (LIANs)

The immune niche formed by local activation of resident cells and response against HCV is characterized by a unique balance between selective pressure by virus-encoded escape variants [27]. Local-active phenotypes in a few intrahepatic CD8 T+ cell clones may reveal the balancing trial of infection, lag-onset immune response targeting a small number, and slow-multiplication period [28].

### 3.1. Lymphoid Organs

During embryogenesis, secondary lymphoid organs like lymph nodes and spleen develop temporally, with mesenteric lymph nodes in the gut and other patches postnatally. Tertiary lymphoid structures develop ectopically in adult organs, organized into B and T cell areas [29]. Cell types were studied in organogenesis and adult lymphoid tissues, focusing on lymphatic vessel interaction, FXIIIA+ fibroblastic reticular reticular cell origin and migration patterns, and T cell apoptosis induction [30].

### 3.2. Mucosal Immune Niches

Mucosal-associated lymphoid tis-

sues (MALT) in the gastrointestinal tract and oral cavity house lymphocytes, with localized MALT being smaller than in the GIT, resulting in a different immune niche. Oral immune foci are expressed in periodontal tissues under inflammatory conditions [31]. Overt oral mucosa inflammation is rare, despite pathogen exposure, and understanding the molecular and cellular mechanisms maintaining tolerance in this challenging tissue is crucial due to its oral environment [32].

Gut-associated lymphoid tissues (GALT) have distinct niches, with abundant sites for inducing immune responses in the intestine. These tissues house B and M cells, which pass antigens to professional antigen-presenting cells (APCs) and other effector sites. Dendritic cells play a crucial role in processing antigens and presenting them to T cells in oral lymphoid foci [33]. Intranasal immunization experiments show the oral cavity connects to cranial and nasal-associated lymphoid tissues (CONALT), which induce immune responses against local antigens. CONALT helps spread activated lymphocytes through mucosal and lymphatic vessels, activating local responses in the GIT [34] (Figure 2).

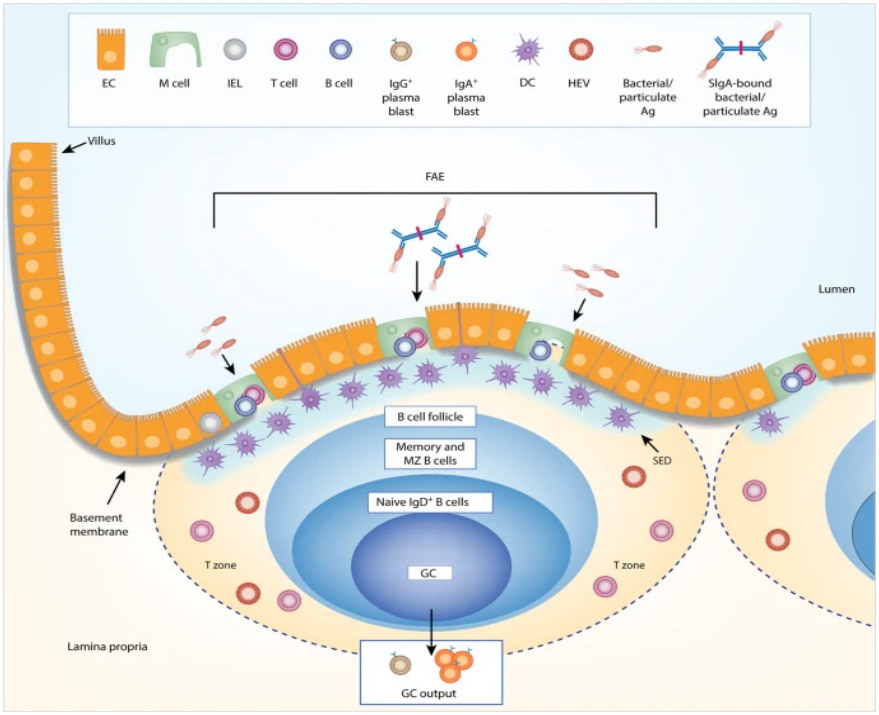


Figure (2): Human gut-associated lymphoid tissues [35].

### 3.3. Tumor Microenvironments

Tumor cells thrive in a complex tumor microenvironment (TME), consisting of hematopoietic, mesenchymal, and extracellular cells. These cells manipulate the TME's components, influencing tumor growth, metastatic progression, and therapy outcomes. Tissue-specific TMEs regulate tumor growth and treatment outcomes [36]. The tumor microenvironment (TME) offers therapeutic options like tumor vaccination, immune checkpoint inhibitors, immuno-metabolism, adoptive cell transfer therapy, and oncolytic viruses. Recent studies focus on heterogeneous tumor tissue aggregates, expanding the therapeutic arsenal against tumors and preventing metastases [37].

Tumor tissues are compressed to extract fresh tissue-based, decellularized, or bioprinted blocks for histology. TME-derived platforms can study immune cell and tumor regulation, with metabolic status playing a role. Broad time-lapsed studies and imaging are needed for stable lesions [38].

## 4. Cellular Components of Immune Niches

Innate immune cells respond to pathogens by identifying conserved structures. Dendritic cells internalize pathogen material, co-stimulate T cells, and B cells encounter T and dendritic cells. Tissue macrophages promote adaptive immune responses, improve pathogen clearance, and produce cytokines to direct and shape innate cell responses [39]. Recent studies examine immune niche composition, functional state of cells, and impact of different types on local responses, challenging and requiring multi-disciplinary efforts to understand immune interaction mechanisms [40].

Spatial organization is crucial for immune protection and inflammation resolution. Computational modeling of immune cell organization can reveal interactions between immune, endothelial, and epithelial cells, enhancing understanding of local immune regulation and disease pathogenesis, especially cancer [41].

### 4.1. T Cells

Unique tissues are colonized by distinct immune cells that protect against

malignant cells, regulate tissue homeostasis, and safeguard against infiltration by aberrant cell types. However, the impact of these specialized immune regulation on local pathology control remains unclear. [42].

T cells are primarily distributed within lymphoid structures, with B zone-located B cells not able to migrate into their T zone counterpart. However, under inflammatory conditions, T cells can migrate into the B zone, becoming effector-memory-like T cells [43].

Three mothers donate interested T cells: active, naïve, and gazing T cells. T cell vocalization is characterized, but how they inquire new tissues during rest is understudied. The search process is thought to be random-walk with unexpected fractal kinetics [44].

**4.2. B Cells**

B cells differentiate into T-cell independent and T-cell dependent pathways, generating high-affinity antibodies and plasma cells. They can form antibody-forming cells in extra-nodal tissues, maintaining lymphotoxin and TNF signaling [45]. Ectopic lymphoid structures have been found in non-obese diabetic mice's salivary glands and in diseases like lupus and rheumatoid arthritis. These structures guide B and T cell infiltration, drive B cell proliferation, and provide B cell support in diseased tissues [46].

T follicular helper-like cells, found outside secondary lymphoid organs, generate antibody class-switched AFCs and secrete IgA. They support germinal centers in response to infections, leading to ectopic germinal centers. Extra-follicular B cell responses may account for IgA secreting cells in mesenteric lymph nodes and gut-associated lymphoid tissues [47]. Tfh-like cells can support B cells in various tissues, but conflicting data exists on their B-cell supporting functions in inflamed human tissue. Non-Tfh cells may help B cells by providing IL-4 and IL-13 for IgE production, potentially linked to allergic diseases and asthma [48]. Effector memory T cells migrate into tissue during acute inflammation, interacting with MHC class II+ antigen presenting cells and ELC expressing Tfh cells to aid antibody production [49].

**4.3. Dendritic Cells**

DCs are specialized antigen-presenting cells that orchestrate the immune response in tissues and at the host/environment interface. They sense

danger signals and mount an immune response, integrating information from tissues and mediating tolerance to self and environmental antigens. [50]. DCs in tissues and lymphoid organs sense danger signals and initiate immune responses, but their histological context is unknown. Ablation of antigen-presenting cell populations in tissues and ocular immunization confirm CD8α+ DCs' role [51]. The study on melanoma vaccination reveals that the absence of CD103+ DC ablation prevents cytotoxic responses priming, but instead, cutaneous CD41+ DCs can mediate anti-tumor immunity. This highlights the importance of DC sub-specialization in tissue studies [52].

### 4.4. Macrophages

Macrophages are the first line of defense in the innate immune system, regulating vital homeostatic tasks like gut duct formation, inflammation resolution, basement membrane remodeling, neuronal maintenance, wound repair, and vasculogenesis [53]. ManR expression often correlates with anti-inflammatory or functional phenotypes. CD206 expression can be found in various cell types. Macrophages are embryonically derived and self-renewing, but their egress and entry mechanisms remain unclear [54].

Recent single-cell transcriptomic technologies reveal macrophage heterogeneity, revealing transitions among stable subpopulations. This information could lead to improved anticancer therapies and understanding of immune suppressor functions, potentially aiding in treating chronic infections or overcoming autoimmunity and transplant rejection [55]. Macrophage populations vary in various tissues, including skin, central nervous system, lungs, and gastrointestinal tract. In the human and murine gut, macrophages show continuous turnover and rapid replacement, indicating their origin from early embryogenesis precursors [56].

## 5. Molecular Signaling in Immune Regulation

Cells in the mesenchymal or epithelial lineage produce and secrete signaling molecules, such as cytokines, chemokines, lipids, and neurotransmitters, to regulate immune cell, tissue, and systemic function. These molecules have specific properties and can mediate their effects through systemic mechanisms [57]. Systemic factors

can range from picogram to nanogram per milliliter, while nonimmune cells use cell surface ligands to engage immune cells. Tumor cells can target these modes of action. Localized immune regulation involves various factors with long-range and short-range effects [58]. Secreted factor production and immune regulation have significant effects on tissues and the body. Pancreatic islet cells' high VSGFs help evade local attack, illustrating immune regulation. However, demonstrating physiological levels of broadly acting secreted factors in in situ immune responses remains a challenge [59].

### 5.1. Cytokines and Chemokines

Cytokines and chemokines are crucial components of immune niches, influencing immune cell proliferation, survival, differentiation, and activation. They regulate leukocyte migration and are small secreted proteins with a diameter of 20 kDa. Chemokines are classified into four families based on cysteine positions and bind to G protein-coupled receptors with seven transmembrane domains [60]. Recent advancements in understanding chemokines' molecular basis and their interactions with receptors have led to classifications based on functional properties. Pro-inflammatory cytokines include IL-1, IL-6, TNF-α, IL-12, and type I and II interferons, while anti-inflammatory and Th2-oriented factors include IL-10, TGF-β, and type III interferons [61]. Chemokines are used in immunotherapeutic strategies to enhance T-cell infiltration into tumors. They are injected locally or systemically, targeting specific tumor cells' sensitivity to T-cell-mediated cytotoxicity. These chemokines are typically administered through nonviral plasmid DNAs or T receptor-mediated migration [62].

### 5.2. Cell-Cell Interactions

Cell-cell interactions are crucial for proper immune responses within tissues due to their high diversity and proximity. This "tissue mosaic" allows immune and supporting cells to interact face-to-face, gathering and disseminating signals. Understanding cell-cell interactions is enhanced by studying histological immunity within tissues or solid organs [63].

Cell-cell interactions can be observed through tissue embedding and sectioning. The tissue sample is embedded into a resin block, then sectioned

into thin slices. Staining may reveal the structure under a light microscope. Direct cell-cell interactions of immune cells demonstrate the importance of spatial arrangement in determining responses [64]. Cell-cell contact facilitates immune activation and suppression, providing complex information to recipient cells. The effect depends on environmental circumstances, such as cell types communicating. Cell surface-associated receptors and immune-regulatory factors affect immune outcomes. Cell-cell interactions can be transient or prolonged, depending on growth or communication needs [65]. Immune responses involve cell-cell interactions, genetic and epigenetic alterations, free diffusion, localized or non-local sources, requiring consideration of each RNFG and its effects due to cell-cell and cell-tissue interactions [66].

### 5.3. Extracellular Matrix Components

The ECM, a network of fibrous and adhesive molecules, is crucial for tissue organization and function. It consists of proteins, glycoproteins, proteoglycans, and polysaccharides. During tissue repair, pathological conditions, or diseases like cancer, ECM remodeling affects cancer and immune cell function [67]. The ECM, besides providing structural support, is a reservoir of signaling compounds involved in healing processes. Matrix-derived factors establish an inflammatory environment, allowing neutrophils and macrophages to recruit for pathological processes. The composition and structure of the ECM influence their adhesion and remodeling during wound healing [68].

## 6. Role of Immune Niches in Disease

Recent histological studies on immune niches in tissues reveal significant progress in understanding local immune regulation and pathology. These studies provide valuable insights into the immune microenvironment, highlighting the importance of understanding immune cell characteristics [69].

Brain-resident immune niches, such as pMOs in the skull bone marrow cavity, regulate innate immunity through tissue-specific interactions. Germinal center-like immune niches promote autoantibody production, leading to brain-specific autoimmune diseases.

Cerebellum and spinal cord immune niches regulate B cell and Treg cell activities, mitigating inflammation and ALS progression [70].

Immune niches may undergo functional reprogramming in response to chronic stimuli like cancer or infections, potentially disrupting tissue homeostasis and affecting systemic immunity. Current studies focus on spatial heterogeneity and co-localization of immune cells, but reprogramming immune niches in non-lymphoid organs could help identify tissue homeostasis disturbances, aid disease progression monitoring, and improve therapeutics [71].

### 6.1. Autoimmune Disorders

Autoimmune diseases like diabetes and RA disrupt immune homeostasis, with Th1 and Th17-related diseases causing tissue destruction. T helper cell subgroups, secreted cytokines, induce inflammation, requiring self-tolerance to prevent pathological immune responses [72]. Autoimmunity is triggered by environmental and genetic factors, including viral infections, pathogens, tissue injury, and smoking, leading to a breakdown of self-tolerance and the development of autoimmune diseases [73].

Tissue-specific autoimmune diseases cause immune homeostasis degeneration in specific target tissues, affecting organs and tissues like pancreas, joints, brain, and gland. Systemic autoimmune diseases progress without target tissue specificity and often correlate with decreased immune tolerance against non-self, high-density self-antigenic components like DNA and ssRNA. Challenges include longer infiltrate times and limited histological techniques [74].

### 6.2. Infectious Diseases

High-resolution imaging techniques have significantly improved understanding of HIV/SIV infections by examining tissue changes and their cellular, histological, and spatial descriptions. This information has led to a new question: do tissue changes mirror those in lymphoid organs, and vice versa? This could help understand immune niche functionality [75]. The question of the difference between chronic and acute lymphoid organ involvement is not trivial due to the different levels of architecture, chronic and acute events, and mechanisms. Deep documentation of patterns and events can bridge this

gap, identifying similarities in progressions and processes [76]. This presentation discusses processes in tissues and lymphoid organs following viral infection, focusing on HIV infection. It highlights similarities between tissues and lymphoid organs, bridging gaps in current knowledge and building upon existing findings. The presentation also anticipates the sharing of tissue histologies to other studies for further impact [77].

### 6.3. Cancer Progression

Solid tumors develop immune tolerogenicity, affecting Treg, which exhausts antitumor CD8 response. Circulatory relocalization of intratumor CMT-93 immPCs provides insights into cellular aspect of this tolerance, compared to gut-associated tissues [78]. Focusing-deficient CMT-93 cells may rent a hypersized immune niche during progressive gout (PG) by amplifying TGFb-rich lymphatics and secreting Vascular Endothelil Growth Factor C. This helps clinically discover tumor-centric PG and immune/effectoric education, allowing for the development of therapeutic strategies for PG [79].

Intercellular signaling oscillators, developed using chemical kinetics, model complex spatiotemporal oscillatory well patterns with distinctive features inconsistent with immune tolerance. These oscillatory dynamics allow for immune-centric *in vivo* experiments and could help develop immunity-targeting therapies, potentially revealing responses to nonsustained immune tolerance [80].

## 7. Techniques for Studying Immune Niches

A niche is an ecosystem that supports growth and flourishing for organisms, including tumors. This concept can help understand immune responses to tumors. Machine learning-based image segmentation was used to study the association of CD8+ T cell infiltration and immunochemical scores with distance survival in esophageal squamous cell carcinoma. The spatial association of CD8+ T cells with different distances had a prognostic ability for survival [81].

Tissue and process microanatomy helps understand local immune responses through histological segregation and mapping tissue compartments. Combining immunohistochemistry and

in situ signaling molecular detection, organs' responses to various immune challenges are unraveled, providing insights into immune niches and their regulation in health and disease, particularly cancer and chronic inflammation [82].

### 7.1. *In Vivo* Imaging

In vitro imaging is a powerful tool for monitoring immune cells and their tissue environments. It visualizes immune and non-immune cells on brain slices from mice, measuring microglia motility and morphological changes in Alzheimer's disease models. Immunohistochemical characterization and super-resolution microscopy are powerful approaches for cellular interactions and dynamics [83]. *In vivo* optical imaging offers advantages such as longitudinal imaging, measuring multiple parameters like motility, morphology, and vessel permeability, and extending knowledge to other experimental systems. However, concerns remain about whether observed observations in in vitro analysis are evident *in vivo*, and sample preparation for disturbance [84]. Advancements in live-imaging technology for bio-inert approaches enable the measurement of cell-cell interaction, protein/molecule interaction, and diffusion behavior. High-promotional frame-rate laser-scanning and fast multiply-phosphors can outlay conventional imagers, making it possible to measure more parameters [85].

### 7.2. Single-Cell RNA Sequencing

Single-cell RNA sequencing (scRNA-Seq) is revolutionizing tissue study by characterizing thousands of cells in a single-cell. Multiplexing and sophisticated computational tools enable targeted experiments, increasing statistical power and enabling biological validation on larger cohorts. Next-generation sequencing technologies are revolutionizing immune cell study, bringing single-cell genomics to users without computational or bioinformatics expertise [86]. This chapter provides an overview of scRNA-Seq technologies and their applications, focusing on practical applications in characterization of rare immune populations. It emphasizes collaboration and specialist expertise for successful experiments, and provides workflow guidelines for sample acquisition, data curation, and analysis. As technologies become more widely available, researchers must identify the best solution [87].

### 7.3. Flow Cytometry

Obtaining cellular material from ex vivo tissues is crucial for flow cytometry analysis. Different tissues require unique digesting conditions, but proteolytic or gelatinase enzymes are common. Cell dissociation can lead to loss of antigen, making fixation necessary. Non-fixative techniques use enzymatic digestion at mild conditions, maintaining temperature between 34.5 and 37°C, incubation time, and pH drop using sodium bicarbonate. These conditions maximize antigen retention and allow 40-60% cell yield recovery [88].

Resonance energy transfer (RET)-based spectral unmixing reduces emission from multiple-fluorescent dyes in multivalent staining. The dyes are optimally selected from a commercial catalogue, and individual calibration is required for each fluorescent aperture. The complexity of data necessitates sophisticated machine-learning approaches for statistical differentiation [89].

## 8. Therapeutic Implications of Immune Niches

Understanding the distribution and organization of immune cells and tissues is crucial for understanding the immune system's protective role without causing harm. Differences in immune cell abundance, type, morphology, and activation level can lead to tissue-specific responses. Tissues have specialized niches for maintaining immune functions, and health and disease states can alter tissue immune organization [90]. The immune system's integration and study as a system remains a challenge. Therapeutic strategies include localized drug delivery, nano- and micro-thermal stress, microbiome remodeling, passive immunization, and locally activated therapy to promote immune health [91].

The study of the immune system is a complex task requiring molecular, cellular, and organ-level research. Advances in imaging, transcriptomics, proteomics, and bioengineering techniques will provide new insights into immune organization and tissue specificity. Collaborations between systems immunologists, histologists, pathologists, and biologists will accelerate

progress. Despite key knowledge gaps, scientists anticipate discoveries impacting human health and well-being [92].

Understanding 2D and 3D visual guidance representations of immune organization is crucial for imaging analysis of immune niches in tissues. Integrating immuno-images and omics will reveal more information about immune organs and tissues in health and diseases. More labs, ethical regulations, and advanced bioengineered imaging equipment are needed [93].

### 8.1. Immunotherapy Strategies

Targeting the tumor microenvironment (TME) may be a successful alternative to vaccinia viruses for enhancing anti-tumor immune responses, focusing on tumor-infiltrating lymphocytes and tumor-residing dendritic cells [94] (Fgure 3).

Therapy effects tumor growth and immunity, but not immune response. Some treatments enhance systemic immunity. Effector T-cell function and interference with Tregs are related. Anti-CTLA-4 monoclonal antibodies may deplete Tregs in the TME, but immune-related auto-reactivity and toxicity have been reported. Tregs help tumors escape immune recognition. CTLA-4 is a critical regulator of T-cell activation [95].

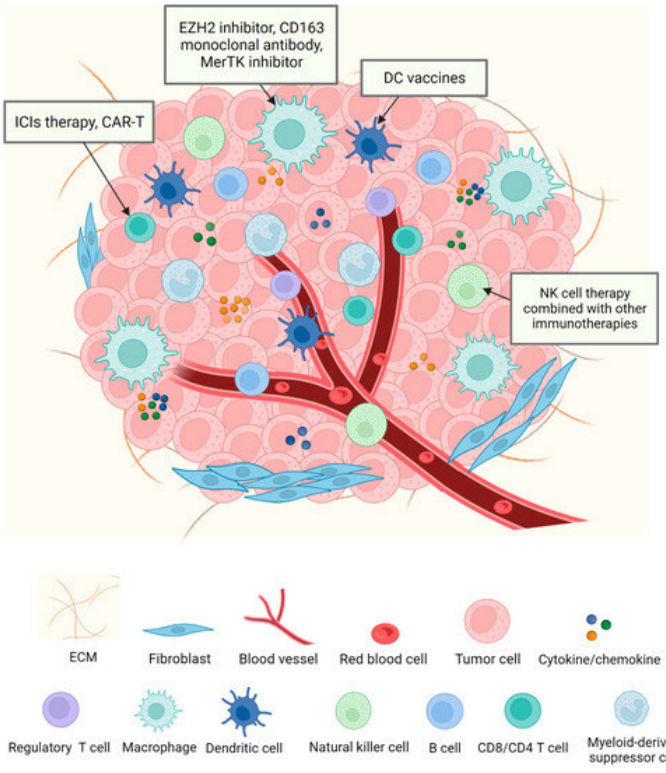


Figure (3): Tumor microenvironment and immunotherapies [96].

### 8.2. Vaccine Development

The vaccination process involves a linear pipeline from pathogen exposure to protective immunity. The innate immune system activates, processing vaccine antigens into immunogenic peptides. Activated cells transport vaccines to lymph nodes, where naïve B and T cells differentiate into memory cells [97].

The vaccine field is increasingly complex, with multiple processes occurring simultaneously or in sequence, potentially skewing the immune response. This raises questions about the sufficiency of current models in understanding successful immune responses. The manuscript presents a sequence of events after pathogen or vaccine exposure, focusing on early events during vaccine delivery [98]. The aim is to highlight the diverse effects of vaccination and promote research in innovative biomaterials. Materials used to deliver vaccines can control immune system physiology, creating desired immune responses. Approaches leverage biomaterials for precise delivery, focusing on prophylactic subunit vaccines and infectious disease prevention [99].

### 8.3. Targeting the Tumor Microenvironment

Cancer is a major global health issue, and immune checkpoint blockade has been developed to combat tumor cells. However, it is often ineffective in most cases. The tumor microenvironment plays a significant role in the immune response to tumors, making it a target for immunotherapy. Targeting the tumor microenvironment could enhance immune response, potentially reactivating immunity against Ewing sarcoma (ES) tumors and making immune checkpoint blockers more effective [100]. The study proposes optimizing agents to target tissue-specific tumor microenvironments and increasing immunity against tumors, resulting in 70% tumor-killing in mice after ES 8-day post-grafting, and targeting CNS cancers for esophageal cancer treatment [101].

## 9. Future Directions in Immune Niche Research

Despite progress in understanding immune regulatory niches, questions remain unexplored. These include whether additional cell types contribute to immune suppression, the range

of targets of immunosuppressive pathways, how immunity is reengaged post-pathology resolution or pathogen release, and the contributions of different cell types or pathways to the overall immune response. Advances in histology, imaging, and proteomics techniques can help address these questions [102]. Pro-inflammatory and type I effector T cell states are detrimental in allergic airway inflammation and chronic graft-versus-host disease. Understanding how these states prevent reactions and how they influence local responses could enhance our understanding of the immune system at the tissue-histological level [103].

### 9.1. Emerging Technologies

Advances in imaging and mass cytometry techniques enable comprehensive tissue visualization, providing a holistic view of the immune system. However, accessibility remains limited, necessitating global investment in infrastructures for diverse biomedical investigations. Multidimensional methodologies, immunohistochemistry, and innovative imaging techniques are crucial for understanding human and simian immunodeficiency virus infections [104]. New approaches are being developed to create and investigate local cellular and biomolecular spatiotemporal microenvironments, providing insights into tissue immune niches and physiological immune regulation. These devices create a vascularized tissue microenvironment that can control exogenously modulate local immune responses through sustained release of on-site biomolecules. This platform, known as the NICHE, opens new avenues for research and clinical applications [105].

## 10. Conclusion

The human immune system is a complex network of specialized cells that protect against pathogens and environmental insults. Recent research using human tissue resources and computational methods has revealed its heterogeneity and how it is influenced by factors like environment, lifestyle, sex, and genetic susceptibility to diseases. Studies have shown immune scars in tissues associated with chronic inflammatory disease, autoimmune conditions, and aging, and age-related susceptibility to severe seasonal influenza. Disparities in heterologous immunity across demographics are linked

to different responses to viruses and vaccines. The immune system protects the body from pathogens, environmental toxins, and cancer. It maintains homeostasis by controlling inflammation and tissue repair. The immune response consists of innate immunity, which provides immediate protection, and adaptive immunity, which generates long-term protective immunity. Understanding this complex system is challenging in humans.

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