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Review

# Engineered Microbes Transforming Immune Modulation Across Cancer, Autoimmunity, and Infection

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## Abstract

Engineered microbes are emerging as a new class of living immunotherapeutics capable of sensing, interpreting, and actively reshaping host immune systems. Unlike conventional biologics or cell therapies with fixed mechanisms of action, engineered microbial platforms operate as dynamic systems that integrate environmental, metabolic, and immunological cues, process these inputs through programmable biological circuits, and execute context-dependent immune modulation with spatial and temporal precision. This review presents an immune-first framework that conceptualizes engineered microbes as distributed immune-computational systems defined by coordinated sensing, signal processing, memory, and effector functions embedded within host immune networks. Organizing the field around immune logic rather than microbial taxonomy or disease category, we examine how engineered microbes detect tissue-specific and immune-state signals, translate these inputs through synthetic processing modules, and generate immune outputs that activate, suppress, educate, or reprogram immunity across cancer, autoimmunity, and infectious disease. We further define immune safety architecture as a core design principle governing inflammatory control, tolerance preservation, adaptive immunity, and therapeutic termination, and discuss the translational and regulatory implications of immune-state-resolved clinical evaluation. Together, this framework positions engineered microbes as programmable immune systems and establishes a unifying conceptual foundation for their development as next-generation living immunotherapies.

**Keywords:** engineered microbial therapeutics; living immunotherapies; immune computation; systems immunology; cancer immunomodulation; immune tolerance engineering; immune safety architecture; immune-state endpoints

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## 1. Introduction: Microbes as a New Class of Immunotherapeutics

The field of immunotherapy has expanded from soluble biologics and small molecules to engineered immune cells and complex biomaterials [1,2]. A parallel and increasingly influential development is the emergence of engineered microbes as programmable agents capable of sensing, interpreting, and reshaping host immunity. These living systems are more than delivery vehicles or microbiome supplements [3,4]. They possess intrinsic immunological functions that can be redesigned, amplified, or redirected through synthetic biology to achieve precise modulation of local and systemic immune responses.

Several features make immunity the central axis through which engineered microbes should be understood. Microbes naturally interact with host immune pathways at nearly every tissue surface. They engage pattern recognition receptors, tune cytokine and chemokine networks, influence antigen presentation, and remodel metabolic landscapes that affect T cell and myeloid cell function [5,6]. When redesigned with synthetic circuits, these immune interactions can be converted into intentional therapeutic programs. Engineered bacteria can sense inflammatory cues, adjust their behavior in

response to environmental signals, and deliver tailored immunomodulatory outputs that reshape specific pathways involved in cancer, autoimmunity, or infection [3,7]. This positions microbes not as passive colonizers but as programmable immune actuators.

The conceptual shift from microbial vectors to microbial immune engineering is essential. Early approaches relied on microbes as carriers for antigens or therapeutic proteins. The new generation of engineered strains incorporates logic gates, feedback circuits, and metabolite-responsive modules that allow real-time regulation of immune activity. These systems can control the intensity, timing, and spatial localization of immune responses with a degree of adaptability that non-living platforms cannot achieve [3,7,8]. For example, bacteria engineered to express pro-inflammatory cytokines only within hypoxic tumor niches can activate cytotoxic immunity while avoiding systemic toxicity. Conversely, strains engineered to promote tolerogenic signals in the gut can suppress inappropriate inflammation without weakening host defense [9–11].

A central advantage of microbial immunotherapeutics is their ability to unify multiple disease contexts under a single technological framework. Cancer requires localized activation of cytotoxic and innate inflammatory pathways [3,11]. Autoimmune and inflammatory diseases require restoration of tolerance, enhancement of regulatory networks, and repair of mucosal barriers [12]. Infectious diseases benefit from targeted augmentation of antimicrobial immunity without provoking immunopathology [13]. Engineered microbes can be adapted to each of these immune states by altering sensing modules, circuit topology, and effector outputs [3,14]. This cross-indication flexibility is distinctive among therapeutic platforms and reflects the modularity of immune pathway architecture itself.

Contemporary immunotherapies remain limited by several constraints that living microbial systems are positioned to overcome. Monoclonal antibodies and cytokine therapies lack spatial precision and often produce systemic adverse effects [15,16]. Cell therapies require complex manufacturing and can be difficult to deliver to mucosal sites or deep tissue microenvironments [17,18]. Small molecules have limited ability to encode conditional or adaptive behavior [19]. Engineered microbes inherently solve many of these challenges. They can autonomously navigate tissue landscapes, respond to microenvironmental cues, sustain therapeutic activity over time, and terminate activity through built-in circuit logic or immune-mediated clearance [20,21]. Their ability to operate continuously within the host, adjusting their activity as immune states evolve, provides a dynamic mode of intervention absent from conventional therapeutics.

This review adopts an immune design logic that organizes the field not by disease category or microbial chassis but by the mechanisms through which engineered microbes interface with immunity. This perspective provides a coherent framework to understand how microbial sensing, signal processing, and effector delivery can be tuned to achieve specific immune outcomes. The following sections examine the design principles of microbial immunotherapeutics, the intrinsic immune behaviors of different microbial platforms, and their application across cancer, autoimmunity, and infectious disease. Together, these concepts illustrate how engineered microbes are transforming immune modulation and establishing a new class of living immunotherapies.

## 2. The Immune Logic of Engineered Microbes as a New Framework for Living Immunotherapies

Engineered microbes function as living computational systems that sense defined features of the host environment, process these signals through synthetic biological circuits, and generate targeted immunological outputs. Their therapeutic value comes not only from the molecules they deliver but from the logic that governs their behavior. Unlike conventional immunotherapies with static modes of action, engineered microbes integrate real-time information about tissue physiology and immune activation, adjusting their responses with precision that is difficult to achieve through non-living platforms [4,22,23]. Understanding these organisms as programmable immune actuators requires a structured framework that links the signals they detect, the genetic circuits they deploy, and the immune pathways they modulate. This section establishes such a framework, defining engineered

microbes through the interplay of immune inputs, synthetic processing modules, and immunological outputs (Figure 1).

<b>Immune-computational logic of engineered microbial immunotherapies</b>		
<p><b>Immune inputs sensed by engineered microbes</b></p> <p><b>Inflammatory cues</b></p> <ul style="list-style-type: none"> <li>• ROS</li> <li>• Nitric oxide</li> <li>• Hypoxia</li> </ul> <p><b>Cytokine signals</b></p> <ul style="list-style-type: none"> <li>• Interferons</li> <li>• TNF</li> <li>• IL-6/IL-10</li> </ul> <p><b>Metabolic cues</b></p> <ul style="list-style-type: none"> <li>• Lactate</li> <li>• Short-chain fatty acids</li> <li>• Tryptophan metabolites</li> </ul> <p><b>Pathogen-associated signals</b></p> <ul style="list-style-type: none"> <li>• Quorum signals</li> <li>• Virulence factors</li> <li>• PAMPs</li> </ul> <p><b>Microbiota and tissue state</b></p> <ul style="list-style-type: none"> <li>• Dysbiosis</li> <li>• Nutrient availability</li> <li>• Barrier disruption</li> </ul> <p>→ <b>Contextual immune information</b></p>	<p><b>Programmable immune computation</b></p> <p><b>Logical integration</b></p> <ul style="list-style-type: none"> <li>• AND / OR gates</li> <li>• Band-pass filtering</li> </ul> <p><b>Feedback regulation</b></p> <ul style="list-style-type: none"> <li>• Positive feedback</li> <li>• Negative feedback</li> </ul> <p><b>Memory modules</b></p> <ul style="list-style-type: none"> <li>• Toggle switches</li> <li>• Recombinase-based memory</li> </ul> <p><b>Population-level logic</b></p> <ul style="list-style-type: none"> <li>• Quorum sensing</li> <li>• Division of labor across strains</li> </ul> <p><b>Spatial control</b></p> <ul style="list-style-type: none"> <li>• Tissue-restricted activation</li> <li>• Micro-niche localization</li> </ul> <p>→ <b>Computed immune response</b></p>	<p><b>Context-dependent immune outputs</b></p> <p><b>Immune activation</b></p> <ul style="list-style-type: none"> <li>• Cytotoxic T cells</li> <li>• NK cell activation</li> <li>• STING pathway activation</li> </ul> <p><b>Immune suppression and tolerance</b></p> <ul style="list-style-type: none"> <li>• Regulatory T cells</li> <li>• IL-10 / TGF-<math>\beta</math></li> </ul> <p><b>Immune education</b></p> <ul style="list-style-type: none"> <li>• Antigen display</li> <li>• Dendritic cell priming</li> <li>• Antigen spreading</li> </ul> <p><b>Immunometabolic rewiring</b></p> <ul style="list-style-type: none"> <li>• Lactate consumption</li> <li>• Adenosine / tryptophan modulation</li> </ul> <p><b>Barrier repair and tissue protection</b></p> <ul style="list-style-type: none"> <li>• Epithelial reinforcement</li> <li>• Tight-junction support</li> </ul> <p>↻ <b>Immune state remodeling</b></p>

**Figure 1. Engineered microbes as distributed immune-computational systems.** Engineered microbial immunotherapies function as living systems that integrate immune-relevant environmental information, process these signals through programmable biological circuits, and generate context-dependent immune modulation. These platforms sense diverse immune inputs, including inflammatory cues, cytokines, metabolic signals, pathogen-associated factors, and microbiota or tissue state, and transform this information through logical integration, feedback regulation, memory formation, population-level coordination, and spatial control. The resulting immune outputs can activate, suppress, educate, or reprogram host immunity through cytokine delivery, antigen presentation, immunometabolic modulation, and barrier repair. Together, these processes form a closed-loop system embedded within host innate and adaptive immune networks, enabling engineered microbes to dynamically sense, compute, and reshape immune system state across disease contexts.

Taken together, these properties position engineered microbes as distributed immune-computational systems rather than passive therapeutic carriers. They continuously sense immune-relevant signals, integrate these inputs through programmable biological logic, store contextual information through circuit-encoded memory, and execute immune-modulatory actions with spatial and temporal precision. In this view, engineered microbes function analogously to immune cells or synthetic immune circuits, implementing sensing, decision-making, memory, and actuation directly within host immune networks. This computational framing provides a unifying logic for understanding how microbial platforms can be rationally designed, evaluated, and deployed across cancer, autoimmunity, and infectious disease. The application of this immune-computational framework to distinct disease contexts, including cancer, autoimmunity, and infection (Figure 2).

<b>Disease-specific immune logic implemented by engineered microbes</b>		
<p><b>Cancer immune logic</b></p> <p><b>Immune inputs</b></p> <ul style="list-style-type: none"> <li>• Hypoxia and lactate-rich metabolism</li> <li>• Tumor cytokines</li> </ul> <p><b>Processing logic</b></p> <ul style="list-style-type: none"> <li>• Conditional activation</li> <li>• Immune amplification</li> </ul> <p><b>Immune outputs</b></p> <ul style="list-style-type: none"> <li>• STING pathway activation</li> <li>• IL-12 delivery</li> <li>• Antigen spreading</li> </ul> <p><b>Outcome</b></p> <ul style="list-style-type: none"> <li>• Inflamed tumor microenvironment</li> <li>• T cell infiltration</li> </ul>	<p><b>Tolerance-oriented immune logic</b></p> <p><b>Immune inputs</b></p> <ul style="list-style-type: none"> <li>• Pro-inflammatory cytokines</li> <li>• Dysbiosis</li> <li>• Epithelial stress</li> </ul> <p><b>Processing logic</b></p> <ul style="list-style-type: none"> <li>• Tolerance-biased computation</li> <li>• Inflammatory attenuation</li> </ul> <p><b>Immune outputs</b></p> <ul style="list-style-type: none"> <li>• IL-10 and TGF-<math>\beta</math></li> <li>• Regulatory T cell induction</li> <li>• Barrier repair</li> </ul> <p><b>Outcome</b></p> <ul style="list-style-type: none"> <li>• Immune re-education</li> <li>• Restoration of tolerance</li> </ul>	<p><b>Immune enhancement logic</b></p> <p><b>Immune inputs</b></p> <ul style="list-style-type: none"> <li>• Pathogen-associated signals</li> <li>• Inflammatory cues</li> </ul> <p><b>Processing logic</b></p> <ul style="list-style-type: none"> <li>• Immune amplification</li> <li>• Trained immunity programming</li> </ul> <p><b>Immune outputs</b></p> <ul style="list-style-type: none"> <li>• Interferon responses</li> <li>• Myeloid cell activation</li> <li>• NK cell activation</li> </ul> <p><b>Outcome</b></p> <ul style="list-style-type: none"> <li>• Enhanced host defense</li> <li>• Pathogen control</li> </ul>

**Figure 2. Disease-specific immune logic implemented by engineered microbes.** Engineered microbial immunotherapies apply a shared immune-computational framework to achieve distinct immunological objectives across disease contexts. In cancer, engineered microbes sense tumor-associated cues such as hypoxia, inflammatory cytokines, and altered metabolism and process this information through conditional activation and amplification logic to drive immune outputs including STING pathway activation, localized cytokine delivery, and antigen spreading, resulting in conversion of immunologically cold tumors into inflamed, T cell-infiltrated microenvironments. In autoimmunity and chronic inflammation, microbial platforms detect inflammatory cytokines, dysbiosis, and epithelial stress and implement tolerance-biased computation that restrains inflammation, promotes regulatory T cell differentiation, delivers IL-10 and TGF- $\beta$ , and reinforces epithelial barrier integrity, leading to immune re-education and restoration of tolerance. During infection, engineered microbes sense pathogen-associated and inflammatory signals and engage immune amplification and trained immunity programs to drive interferon responses, activate myeloid and natural killer cells, and enhance host defense and pathogen control. Together, these examples illustrate how a unified immune-computational architecture can be selectively tuned to activate, suppress, or enhance immunity according to disease-specific immune requirements.

### 2.1. Immune Inputs and the Signals Engineered Microbes Sense

Engineered microbes rely on their ability to detect the biochemical and immunological signatures that define specific tissue states. These sensory inputs determine when and where a microbe should activate a therapeutic program. Inflammation-associated molecules provide important contextual information. Reactive oxygen species, nitric oxide, and hypoxic conditions are characteristic of tumors, inflamed mucosa, and infected tissues [20,23–25]. Microbes naturally possess sensing systems for oxidative and nitrosative stress, and these can be redirected to control the activation of therapeutic circuits [24,26]. Hypoxia-responsive promoters can restrict immune-stimulatory outputs to tumor cores, while nitric oxide sensors can initiate anti-inflammatory functions at sites of mucosal damage [27,28]. Such environmental signals give engineered microbes spatial and contextual precision.

Host cytokines serve as another major category of immune input. These molecules encode dominant immune states ranging from acute inflammation to regulatory tolerance. Engineered microbes can detect cytokines such as interferons, TNF, IL-6, or IL-1 family members through synthetic receptors or transcriptional sensors [4,23,27]. This allows microbes to adjust their behavior according to the surrounding immune landscape. In cancer, inflammatory cytokines can be used as triggers for local immune activation [11,29], while in autoimmune disease the same molecules can initiate circuits that reduce inflammation or promote tolerance [30]. Cytokine sensing enables microbes to behave as adaptive elements within immune networks rather than simple vehicles of therapeutic molecules [31].

Metabolic cues provide a further layer of immune-relevant information. Metabolites such as lactate, short-chain fatty acids, tryptophan derivatives, and bile acids are tightly linked to specific immune phenotypes [5]. High lactate concentrations reflect immunosuppressive tumor niches and can be used to activate circuits that enhance antigen presentation or promote cytotoxic responses [32]. SCFAs and tryptophan metabolites influence Treg and Th17 differentiation, offering opportunities to engineer microbes that stabilize immune tolerance [33]. By sensing metabolites and in some cases chemically transforming them, engineered microbes can directly influence immunometabolic pathways that regulate T cell and myeloid function [9].

Inputs can also originate from pathogens. Microbes can detect pathogen-associated signals such as quorum-sensing molecules, virulence factors, or cell wall components [34,35]. These signals can trigger engineered bacteria to deliver immune stimulants that recruit phagocytes or enhance innate responses. Detection of pathogen-associated cues situates engineered microbes within the host's broader defense architecture and enables targeted amplification of antimicrobial immunity only where needed.

Finally, engineered microbes can sense features of the microbiota itself. Patterns of microbial composition, nutrient availability, or interspecies metabolites indicate whether mucosal ecosystems are balanced or perturbed [31,36]. Dysbiosis can therefore serve as an activation signal for circuits that reinforce barrier function, secrete tolerogenic mediators, or restore colonization resistance [6,37]. In this way, engineered strains can participate in the preservation of immune homeostasis and intervene at early stages of disease development.

Together, these inputs allow engineered microbes to operate as context-aware immune modulators. They respond selectively to pathological conditions rather than acting indiscriminately. They sense inflammation, immune activation, metabolic stress, pathogen presence, or microbiota imbalance, and they convert these signals into defined immunological actions. This sensory layer forms the foundation of the immune logic that shapes the behavior of living microbial therapeutics.

## 2.2. Processing Modules and the Interpretation of Immune-Relevant Signals

Once immune inputs are detected, engineered microbes rely on internal processing modules that determine how those signals are interpreted and translated into action. These modules constitute the computational core of microbial immunotherapeutics. They allow microbes to make logical decisions, regulate amplitude and timing of responses, and coordinate multicellular behavior [3,8,22,23]. Their design draws heavily on synthetic biology but aligns with principles found in immune cell signaling, where signal integration, feedback regulation, and memory shape functional outcomes.

Logical gate systems form one of the foundational modules. AND gates allow microbial responses only when multiple conditions are simultaneously met, such as inflammation plus hypoxia or cytokine elevation plus a tumor-specific metabolic cue. This architecture improves specificity and prevents off-target immune activation [23,38,39]. OR gates, by contrast, allow outputs when any of several pathological signals are present. Using combinations of these modules, engineered microbes can execute decision-making programs that mirror the conditional activation seen in T cells or antigen-presenting cells [3,38].

Feedback and negative feedback controls modulate response stability and prevent runaway activation. Positive feedback can amplify weak signals to ensure robust therapeutic action within

pathological niches. Negative feedback can limit excessive production of cytokines or immune ligands, protecting against toxic inflammatory overshoot [3,14,40]. These regulatory modules parallel the natural homeostatic loops that govern immune activation and tolerance, giving engineered microbes the capacity to shape immunological dynamics rather than deliver static stimuli.

More complex circuit designs incorporate band-pass filters that activate only within a defined range of signal intensity. This prevents responses at very low or very high levels of environmental cues, which is particularly important in tissues where gradients of cytokines, metabolites, or oxygen reflect distinct immune states [41–43]. Band-pass logic enables nuanced behaviors such as targeting premalignant inflammatory foci without activating in healthy tissue or responding only within the immunosuppressive threshold of a tumor microenvironment.

Memory modules, including toggle switches or recombinase-based circuits, allow microbes to record prior exposure to specific cues and maintain altered states over time. This property enables persistent immune modulation even after the initial trigger disappears. Microbial memory can support long-term tolerance induction, sustained anti-tumor activity, or prolonged metabolic reprogramming of immune cells [31,44–46]. By embedding memory into microbial behavior, engineered strains can achieve a temporal dimension of immunotherapy that resembles trained immunity in innate cells or long-lived responses in adaptive immunity.

Quorum-sensing networks, a natural communication system among bacteria, can be repurposed to coordinate collective behavior across microbial populations. These systems allow microbes to regulate output intensity based on population density, synchronize therapeutic activities, or create spatially patterned responses [47–49]. Quorum sensing also enables division of labor, where different subsets of microbes produce complementary immune factors only when an entire community is present within the lesion or mucosal environment.

Spatial logic further refines microbial therapeutic action. Engineered microbes can form biofilms or microcolonies in defined tissue regions, allowing localized immune modulation. Circuits responsive to mucosal adhesion molecules, epithelial glycan patterns, or gradients of oxygen and metabolites can position microbes at the exact sites where immune intervention is needed [22,50,51]. Spatial logic is particularly valuable in mucosal diseases, where microbe localization determines whether cytokines or tolerogenic factors engage epithelial cells, stromal cells, or resident immune populations.

Together, these processing modules form the computational architecture through which engineered microbes translate complex environmental information into precise therapeutic actions. They enable living therapeutics to behave less like passive vectors and more like programmable immune cells that adjust their function according to evolving tissue states.

### 2.3. Immune Outputs and the Modulation of Host Immunity

The final tier of microbial immune logic involves the therapeutic outputs that engineered microbes generate to influence host immune pathways. These outputs define the immunological phenotype that each microbe produces, spanning responses that promote cytotoxic activation as well as those that reinforce regulatory tolerance [3,11,31,35]. When these outputs are integrated with the sensory and processing modules described above, engineered microbes are able to exert highly targeted effects on immune networks relevant to cancer, autoimmunity, and infection.

A major class of outputs includes cytokines and chemokines. Microbes engineered to secrete molecules such as IL-12, IL-2, GM-CSF, or IFN family members can activate cytotoxic immunity within tumors or infected tissues. Localized microbial production of Th1 or NK-activating cytokines can convert immunologically cold tumors into inflamed lesions capable of supporting T cell infiltration and antigen spreading [28,52,53]. Conversely, secretion of IL-10 or TGF- $\beta$  can suppress excessive inflammation, promote regulatory T cell differentiation, and restore mucosal tolerance in autoimmune disease [54,55]. These cytokine programs provide high potency with low systemic exposure because engineered microbes confine their activity to defined microenvironments.

Antigen display systems represent another powerful immunological output. Microbes can present tumor antigens, autoantigens, or pathogen-derived epitopes on their surface or within secreted vesicles. These displays can drive antigen-specific T cell priming, enhance cross-presentation by dendritic cells, or induce tolerance depending on the co-delivered signals [56–58]. Antigen display enables microbial therapeutics to function as living vaccines or as tools for restoring immune recognition in cancer and chronic infection.

Expression of engineered immune ligands extends microbial influence to co-stimulatory and co-inhibitory pathways that shape T cell activity. Microbes can be engineered to express PD-L1 decoys that sequester inhibitory signals, or ligands such as CD40L, OX40L, and 4-1BBL that enhance T cell activation [53,59,60]. These ligands can remodel T cell dynamics within tissue niches, reversing exhaustion or enhancing cytotoxic potential without requiring systemic administration of immune agonists. Microbial delivery of these molecules provides spatial precision and reduces toxicity often associated with systemic checkpoint or co-stimulatory therapies.

Tolerogenic factors constitute a distinct therapeutic class especially relevant to autoimmune diseases and inflammatory disorders. Engineered microbes can produce IL-10, TGF- $\beta$ , retinoic acid, or specialized immunoregulatory metabolites to promote regulatory T cell expansion, stabilize tolerogenic dendritic cells, and repair mucosal barriers [61–63]. These outputs directly counteract pathogenic inflammation and restore homeostatic immune balance in the gut, skin, and other barrier tissues.

Metabolic rewiring is an emerging category of immune output. Many immune responses depend on metabolic pathways related to arginine, tryptophan, adenosine, or lactate. Engineered microbes can consume immunosuppressive metabolites such as lactate within tumors, degrade adenosine to relieve T cell inhibition, or regulate tryptophan pathways that control Treg and Th17 differentiation [64–67]. By reshaping metabolic microenvironments, microbes can influence immune cell function in ways that traditional cytokine therapies cannot achieve.

Together, these outputs demonstrate the versatility of engineered microbes as immunomodulatory agents. They can locally activate, suppress, educate, or redirect immunity depending on their design. When combined with the input detection and processing modules described earlier, these outputs complete a full systems logic through which engineered microbes can be rationally programmed as living immunotherapies tailored to distinct disease contexts (**Table 1**) (**Figure 1**).

**Table 1.** Immune inputs, processing logic, and immune outputs implemented by engineered microbes.

Immune input type	Sensing mechanism	Processing logic	Immune output	Disease context
Hypoxia	Hypoxia-responsive promoters	AND gate with inflammatory cues	IL-12 delivery	Cancer
Tumor-associated cytokines	Cytokine-responsive transcriptional sensors	Signal amplification	STING pathway activation	Cancer
Lactate-rich metabolism	Metabolic sensing modules	Conditional activation	Antigen spreading	Cancer

Pro-inflammatory cytokines	Cytokine sensors (e.g., TNF, IL-6)	Tolerance-biased computation	IL-10 secretion	Autoimmunity
Dysbiosis	Microbiota-state sensing	Inflammatory restraint	Regulatory T cell induction	Autoimmunity
Epithelial stress	Stress-responsive promoters	Feedback attenuation	Barrier repair	Autoimmunity
Pathogen-associated molecular patterns	PRR-based sensing	Immune amplification	Interferon responses	Infection
Pathogen quorum signals	Quorum-sensing receptors	Population-level coordination	Myeloid cell activation	Infection
Inflammatory cues	Cytokine and metabolite sensing	Trained immunity programming	NK cell activation	Infection
SCFAs	Metabolic sensors	Tolerance logic	Regulatory T cell expansion	Autoimmunity
Nitric oxide	Redox-sensitive sensors	Band-pass filtering	Local immune modulation	Cancer
Microbiota nutrient state	Nutrient availability sensing	Adaptive regulation	Immune homeostasis	Chronic inflammation

This immune-computational framing also highlights a growing misalignment between living immunotherapeutics and existing regulatory paradigms. Current approval frameworks remain largely optimized for static agents defined by fixed molecular composition, dose–exposure relationships, and linear pharmacokinetics. Engineered microbes instead act by reshaping immune system state over time, with therapeutic efficacy and risk emerging from dynamic immune network reorganization rather than from single-target engagement. As a result, conventional endpoints such as peak exposure, systemic concentration, or isolated biomarker suppression often fail to capture the true mechanism of action of these systems. Immune-state–resolved endpoints, including cytokine topology, regulatory balance, spatial immune architecture, and memory formation, are therefore not ancillary measurements but central descriptors of both efficacy and safety. Explicitly articulating immune architecture and control logic is likely to become essential for regulatory evaluation, providing a common language through which dynamic, adaptive, and self-limiting living therapies can be meaningfully assessed.

### 3. Microbial Chassis Platforms and Their Intrinsic Immune Personalities

Engineered microbes are often classified according to taxonomic lineage or ease of genetic manipulation. However, for immunotherapeutic applications, a more informative organizing principle is the intrinsic immune personality of the microbial chassis. Each microbial species enters the host with a characteristic pattern of immune recognition, tissue localization, metabolic interaction, and signaling consequence [56,68,69]. These native immune behaviors fundamentally constrain and enable the types of therapeutic programs that can be safely and effectively implemented.

Classifying microbial chassis by immune behavior rather than taxonomy reveals why certain strains are naturally suited for tolerogenic applications while others are predisposed to inflammatory immune activation. Commensal organisms that have co-evolved with host mucosal immune systems tend to reinforce barrier integrity and immune tolerance [70,71]. In contrast, attenuated pathogens retain strong immunostimulatory capacities that can be harnessed for tumor targeting and antimicrobial immunity [22]. Beyond single strains, engineered microbial consortia introduce an additional layer of immune complexity by enabling distributed immune control across multiple microbial actors [72].

This immune-centric framework provides a rational basis for selecting, engineering, and deploying microbial platforms according to desired immunological outcomes rather than convenience of genetic engineering alone. These intrinsic immune personalities define the baseline inflammatory risk, regulatory capacity, and therapeutic suitability of different microbial chassis, independent of taxonomic classification (**Figure 3**).

<b>Intrinsic immune personalities of microbial chassis</b>		
<p><b>Commensal chassis</b></p> <p><b>Immune personality</b></p> <ul style="list-style-type: none"> <li>• Low PRR activation</li> <li>• Tolerogenic bias</li> </ul> <p><b>Biological features</b></p> <ul style="list-style-type: none"> <li>• Mucosal localization</li> <li>• Long-term colonization</li> </ul> <p><b>Therapeutic suitability</b></p> <ul style="list-style-type: none"> <li>• Chronic immune modulation</li> <li>• Tolerance induction</li> </ul>	<p><b>Attenuated pathogenic chassis</b></p> <p><b>Immune personality</b></p> <ul style="list-style-type: none"> <li>• High PRR activation</li> <li>• Pro-inflammatory bias</li> </ul> <p><b>Biological features</b></p> <ul style="list-style-type: none"> <li>• Inflammation-driven tumor and infection-site tropism</li> <li>• Strong innate immune engagement</li> </ul> <p><b>Therapeutic suitability</b></p> <ul style="list-style-type: none"> <li>• Immune amplification</li> <li>• Acute immune activation</li> </ul>	<p><b>Engineered microbial consortia</b></p> <p><b>Immune personality</b></p> <ul style="list-style-type: none"> <li>• Distributed immune control (<i>division of sensing and effector functions across strains</i>)</li> <li>• Balanced activation and regulation (<i>quorum-sensing-coordinated cytokine output</i>)</li> <li>• Programmable PRR engagement (<i>partitioned across strains</i>)</li> </ul> <p><b>Biological features</b></p> <ul style="list-style-type: none"> <li>• Division of labor across strains</li> <li>• Coordinated population-level behavior (quorum sensing, population thresholds)</li> </ul> <p><b>Therapeutic suitability</b></p> <ul style="list-style-type: none"> <li>• Network-level immune modulation</li> <li>• Enhanced robustness and safety</li> </ul>

**Figure 3. Intrinsic immune personalities of microbial chassis.** Microbial chassis differ fundamentally in their baseline immune behaviors, which constrain and enable distinct immunotherapeutic applications independent of genetic payload. Commensal chassis exhibit low pattern recognition receptor (PRR) activation and a tolerogenic immune bias shaped by co-evolution with host mucosal immunity, making them well suited for chronic immune modulation and tolerance induction. Attenuated pathogenic chassis retain strong innate immune stimulatory capacity, characterized by high PRR engagement and pro-inflammatory signaling, which can be exploited for immune amplification in cancer and infectious disease settings but requires stringent safety control. Engineered microbial consortia distribute sensing, processing, and immune output functions across multiple strains, enabling division of labor, coordinated population-level behavior, and layered safety architectures that support network-level immune control. Together, these intrinsic immune personalities demonstrate that therapeutic suitability is determined by immune behavior rather than microbial taxonomy, underscoring the importance of chassis selection as a primary design decision in living immunotherapy.

### 3.1. Commensal Chassis and Their Tolerogenic Immune Profiles

Commensal bacteria such as *Lactobacillus*, *Bacteroides*, and members of the *Clostridia* class exhibit immune behaviors that are strongly biased toward tolerance, homeostasis, and barrier support. These organisms establish long-term residence at mucosal surfaces and have evolved to coexist with the immune system without provoking sustained inflammation [70,73,74]. Their surface structures, secreted metabolites, and metabolic byproducts engage host immune pathways in ways that favor regulatory immune tone rather than defensive activation.

A defining feature of these commensal chassis is their capacity to promote mucosal tolerance. Many *Clostridia* species induce regulatory T cell differentiation through the production of short-chain fatty acids and through epigenetic modulation of immune cells within the lamina propria [75,76]. *Bacteroides* species can stimulate tolerogenic dendritic cell phenotypes and support interleukin-10–dominated immune responses [70,77]. *Lactobacillus* strains modulate epithelial signaling and reinforce tight junction integrity, limiting inflammatory translocation of luminal antigens [78]. These properties make commensal microbes naturally aligned with therapeutic strategies aimed at dampening pathological inflammation rather than amplifying immune activation.

Commensal chassis also interface closely with antigen sampling and IgA-mediated immune regulation. They actively shape the education of mucosal B cells, influence the specificity and breadth of secretory IgA repertoires, and regulate antigen presentation through interactions with specialized epithelial and dendritic cell subsets [79–81]. By occupying niches at the interface between the microbiota and the immune system, commensals play a central role in calibrating immune recognition of luminal antigens. When engineered to display defined antigens or secrete specific immunomodulators, these organisms can leverage existing IgA and mucosal antigen-sampling pathways to induce either antigen-specific tolerance or controlled immune deviation [82].

These attributes confer unique advantages for applications in autoimmunity and inflammatory bowel disease. In such settings, therapeutic goals center on restoring immune balance, reinforcing epithelial barriers, suppressing aberrant effector responses, and expanding regulatory cell populations. Engineered commensal microbes can deliver tolerogenic cytokines, regulatory metabolites, or disease-relevant antigens within a biological context that already favors immune restraint. Their intrinsic low inflammatory profile reduces the risk of systemic immune activation, making them especially well-suited for chronic immune modulation in the gut and other mucosal tissues.

### 3.2. Attenuated Pathogens and Their Pro-Inflammatory Immune Architectures

Attenuated pathogenic bacteria such as *Salmonella*, *Listeria*, *Shigella*, and *Escherichia coli* Nissle retain strong immunostimulatory properties even after virulence attenuation. Unlike commensal chassis that favor immune tolerance, these organisms are intrinsically wired to activate innate immune surveillance pathways and to provoke robust inflammatory responses [83]. This immune personality makes them uniquely suited for applications in which strong immune activation is required, particularly in cancer immunotherapy and in the control of difficult infections [84].

A defining feature of attenuated pathogens is their high inflammatory capacity. Their cell wall components, flagellar proteins, secretion systems, and nucleic acids are efficiently detected by pattern recognition receptors expressed on epithelial cells, macrophages, dendritic cells, and innate lymphoid populations [85]. Engagement of Toll-like receptors such as TLR4 and TLR5, cytosolic DNA sensors linked to the STING pathway, and additional inflammasome-associated receptors results in rapid induction of pro-inflammatory cytokines, type I interferons, and chemokines that recruit and activate effector immune cells [86,87]. Even when virulence factors are weakened, these organisms retain the molecular signatures required to trigger strong host defense pathways.

This intrinsic propensity for immune activation provides important advantages for cancer applications. Attenuated *Salmonella* and *Listeria* preferentially accumulate within tumors due to aberrant vasculature, immune suppression, and necrotic tissue architecture. Once localized, their natural ability to trigger innate immune signaling can convert immunologically silent tumors into

inflamed microenvironments that support antigen presentation, T cell infiltration, and cytotoxic effector function [28,88,89]. When further engineered to deliver cytokines, immune ligands, or tumor antigens, these microbes act as localized immune amplifiers capable of reshaping tumor immunity without inducing widespread systemic inflammation.

Attenuated pathogens are also well suited for infectious disease models in which immune augmentation is desirable [90]. Their strong engagement of innate immune sensors enhances antimicrobial effector pathways, promotes robust adaptive immune priming, and supports durable immune memory [91]. Engineered derivatives of these organisms can be programmed to secrete cytokines that boost macrophage bactericidal activity, enhance interferon signaling for antiviral defense, or recruit neutrophils to sites of persistent infection [92]. In this context, the inflammatory immune personality of the chassis is a therapeutic asset rather than a liability.

Nevertheless, the same properties that make attenuated pathogens powerful immune activators also impose constraints. Excessive inflammation, systemic cytokine release, and unintended tissue damage represent persistent risks. Effective clinical deployment therefore depends on precise attenuation strategies, stringent control of microbial replication, and integration of safety circuits that limit immune activation to defined spatial and temporal windows [22,93]. When these safeguards are properly implemented, attenuated pathogens offer a uniquely potent platform for immune amplification where strong activation is therapeutically required.

### 3.3. Consortium-Based Engineered Communities and Distributed Immune Control

Beyond single-strain platforms, engineered microbial consortia introduce a higher-order level of immune regulation by distributing therapeutic functions across multiple microbial species. In these systems, distinct strains are programmed with complementary sensing, processing, and effector roles, enabling collective behaviors that surpass the capabilities of any individual organism [72,94,95]. This distributed architecture mirrors key features of immune system organization, in which multiple cell types cooperate to generate coordinated responses.

A central advantage of consortia is the division of labor among strains. One strain may specialize in sensing inflammatory metabolites, another in antigen display, and a third in cytokine or tolerogenic factor production. By separating these tasks, each microbial component can be optimized for a specific function without overburdening cellular resources [96]. This modularity allows complex immune programs to be assembled from simpler biological parts and provides flexibility in reconfiguring therapeutic outputs for different disease contexts [48,97].

Consortium designs also enable multi-output immune orchestration. Rather than delivering a single immune signal, a coordinated community can release balanced combinations of cytokines, chemokines, metabolic modulators, and immune ligands. Such multi-dimensional immune programming is particularly valuable in diseases where pathological states arise from network-level dysregulation rather than single molecular defects [98–100]. In cancer, consortia can simultaneously promote antigen presentation, relieve immune suppression, and enhance effector recruitment. In autoimmunity, they can combine barrier repair, regulatory T cell induction, and suppression of pro-inflammatory cytokine axes within a unified therapeutic program [101].

Consortia further provide enhanced robustness and safety. Functional redundancy among strains can buffer against stochastic loss of individual members, while cross-regulatory quorum circuits can coordinate population-level activity and prevent uncontrolled expansion [94]. Importantly, safety features can also be distributed, with certain strains dedicated to containment, self-limitation, or immune-dependent clearance [102,103]. This architecture reduces the likelihood that any single genetic failure compromises the overall safety of the system.

Through division of labor, coordinated immune orchestration, and layered safety, consortium-based platforms extend the immune logic of engineered microbes from single-cell decision-making to community-level computation [104–106]. They represent a critical step toward therapeutic ecosystems capable of sustained, adaptive, and highly regulated immune modulation.

#### 4. Engineered Microbes for Cancer Immunomodulation

Engineered microbes have emerged as a powerful class of cancer immunotherapeutics because of their unique ability to integrate tumor-specific environmental cues with programmable immune outputs [28,107]. Unlike conventional cancer therapies that rely on systemic drug exposure or ex vivo cell manipulation, microbial systems operate directly within the tumor microenvironment. This enables immune modulation to occur precisely where malignant cells evade immune surveillance [84]. The defining feature of this approach is not microbial presence within tumors alone, but the capacity to execute context-dependent immune activation that reshapes tumor-immune interactions with spatial and temporal control [108].

Tumor-homing behavior provides the foundational targeting layer for microbial immunotherapy. Solid tumors present abnormal vasculature, hypoxia, necrotic regions, and immunosuppressive metabolic conditions that preferentially support microbial accumulation [109,110]. Engineered bacteria exploit these features through both passive trapping and active chemotaxis toward tumor-derived metabolites. However, tumor homing alone is insufficient for therapeutic efficacy [3,84]. Its true value lies in enabling highly localized immune modulation that avoids the systemic toxicities associated with traditional cytokine therapies. When tumor localization is linked to conditional immune activation circuits, microbial therapies achieve spatial precision that few other platforms can match [3,84,110].

One of the most potent immune activation strategies enabled by engineered microbes is targeted engagement of the STING pathway [111,112]. The cGAS-STING axis plays a central role in innate immune detection of cytosolic DNA and in the induction of type I interferon responses critical for antitumor immunity. Engineered microbes can be programmed to synthesize and release cyclic dinucleotide STING agonists selectively within tumors. This local activation stimulates dendritic cell maturation, enhances cross-presentation of tumor antigens, and promotes recruitment of cytotoxic lymphocytes [113,114]. Unlike systemic STING agonist delivery, which is limited by severe inflammatory toxicity, microbial delivery confines activation to the tumor microenvironment and allows controlled signal amplitude.

Localized expression of cytokines represents another key dimension of microbial cancer immunotherapy. Cytokines such as IL-2, IL-12, and GM-CSF are among the most powerful immune activators known, yet their systemic use is severely constrained by life-threatening toxicities [115]. Engineered microbes circumvent this limitation by producing these cytokines only within tumors through hypoxia-responsive or inflammation-responsive promoters. Local IL-12 expression promotes Th1 polarization and interferon-dependent antitumor immunity [116]. IL-2 enhances intratumoral T cell expansion, while GM-CSF stimulates dendritic cell recruitment and antigen presentation [117]. The result is a focused immune amplification confined to the malignant niche.

Microbial immunotherapy also enables efficient antigen spreading, a critical requirement for durable cancer immunity. Tumor necrosis induced by microbial inflammation exposes a broader repertoire of tumor antigens to the immune system. Engineered microbes further enhance this process by delivering adjuvants, cytokines, and antigen-presentation scaffolds that support cross-priming [56,118]. This promotes diversification of T cell responses beyond a single dominant epitope and reduces the likelihood of immune escape through antigen loss [119]. Antigen spreading is a key mechanism through which localized microbial therapy generates systemic and long-lasting antitumor immune memory [120].

A particularly innovative application of engineered microbes is the direct expression of immune checkpoint modulators within tumors. Rather than administering systemic antibodies against PD-1, PD-L1, CTLA-4, or related pathways, microbes can be engineered to secrete checkpoint nanobodies or decoy receptors locally [56,118,121]. This approach uncouples therapeutic benefit from systemic immune dysregulation. Local sequestration of inhibitory ligands enhances effector T cell activity specifically within the tumor, while minimizing autoimmune complications in peripheral tissues. Microbial checkpoint modulation thus reframes immune checkpoint blockade as a spatially constrained intervention [11].

Cold tumors characterized by immune exclusion, low antigen presentation, and suppressive myeloid populations represent one of the most difficult challenges in oncology. Engineered microbes offer a direct solution by reconditioning these immunologically silent microenvironments [67]. Through combined delivery of STING agonists, pro-inflammatory cytokines, and metabolic modulators, microbes can convert cold tumors into inflamed lesions that support dendritic cell infiltration, lymphocyte trafficking, and effector function [87]. This immune reprogramming function is distinct from direct tumor cell killing and instead focuses on reconstructing the immune architecture of the tumor microenvironment [56].

Microbial modulation of tumor metabolism further amplifies immunotherapeutic efficacy. Tumors accumulate lactate, adenosine, and kynurenine that suppress cytotoxic immunity and promote regulatory cell populations [65]. Engineered microbes can be programmed to degrade these immunosuppressive metabolites directly within tumors. Lactate consumption relieves T cell exhaustion [122]. Adenosine degradation removes a potent inhibitory signal on lymphocytes. Interference with tryptophan metabolism alters regulatory T cell differentiation [66]. Through these mechanisms, microbes reshape the biochemical terrain of the tumor to favor immune dominance.

The precision of microbial cancer immunotherapy arises from the integration of sensing, processing, and output logic rather than from brute-force immune activation [3]. Tumor-associated hypoxia, inflammatory metabolites, and cytokines serve as input signals that gate microbial activity [123,124]. Genetic circuits implement conditional expression of immunostimulatory payloads. Feedback control prevents excessive cytokine release. This creates a quantitative immune control system embedded directly within the tumor [52]. Such programmable control distinguishes microbial platforms from all existing immunotherapeutic modalities.

Importantly, microbial cancer immunotherapy can operate synergistically with established treatment modalities. Local microbial immune activation sensitizes tumors to immune checkpoint inhibitors by increasing antigen presentation and effector infiltration. It enhances the immunogenicity of radiotherapy and chemotherapy by amplifying damage-associated molecular patterns. It also complements adoptive cell transfer by conditioning the tumor niche to support transferred T cells [118,125–127]. These interactions position engineered microbes not only as standalone immunotherapies but as immune amplifiers for combinatorial oncology.

Safety remains a defining constraint for all cancer-directed immune activation strategies [123]. The same inflammatory capacity that enables tumor destruction also carries the risk of systemic immune toxicity. Engineered microbes address this challenge through multi-layered attenuation strategies, kill switches, metabolic dependencies, and immune-mediated clearance circuits [103,128]. Importantly, the immune system itself becomes part of the safety architecture. As tumors are eliminated and inflammatory cues dissipate, microbial activation diminishes and the organisms are cleared, providing an intrinsic self-limiting feature absent from many drug-based platforms [129].

Together, these properties redefine how microbial therapies function in oncology. Their primary role is not tumor colonization or cytotoxic delivery, but precision immune activation driven by programmable biological logic. By transforming tumors into sites of controlled innate and adaptive immune engagement, engineered microbes establish a fundamentally new paradigm for cancer immunomodulation that is spatially restricted, dynamically regulated, and adaptable across tumor types.

## 5. Engineered Microbes for Autoimmunity and Mucosal Immune Tolerance

Autoimmune and chronic inflammatory diseases arise from persistent failures of immune tolerance rather than from simple immune deficiency [70,130]. In these conditions, the immune system retains its capacity for activation but loses the ability to restrain destructive effector responses against self or harmless environmental antigens. Unlike cancer, where immune amplification is the primary objective, autoimmunity requires precise suppression, redirection, and re-education of immune networks. Engineered microbes are uniquely suited for this task because they operate at mucosal interfaces where immune tolerance is physiologically established and maintained [131–133].

Their ability to deliver localized regulatory signals in a continuous and adaptive manner positions them as an entirely new class of tolerogenic immunotherapies.

One of the most direct strategies for microbial tolerance induction is the engineered secretion of immunoregulatory cytokines such as IL-10 and TGF- $\beta$ . These cytokines play central roles in limiting effector T cell activation, promoting regulatory T cell differentiation, and stabilizing tolerogenic dendritic cell phenotypes [134–136]. When delivered systemically, both molecules are associated with broad immunosuppression and unacceptable side effects. Engineered microbes overcome this limitation by confining cytokine release to mucosal tissues, particularly the intestinal lamina propria. This localized delivery supports immune regulation at disease-relevant sites while preserving systemic immune competence.

In addition to soluble cytokines, engineered microbes can release extracellular vesicles enriched in tolerogenic signals [137,138]. These vesicles can carry regulatory cytokines, microRNAs, and metabolites that influence antigen-presenting cells and lymphocyte differentiation [139]. Vesicle-mediated delivery allows microbes to exert immunomodulatory effects without direct physical contact with immune cells and provides a mechanism for extending regulatory signals deeper into mucosal tissues [140,141]. This form of indirect communication further expands the spatial reach of microbial tolerance programs.

Antigen-specific tolerance represents one of the most important and underdeveloped goals in autoimmune therapy [142–144]. Instead of globally suppressing immunity, the aim is to silence only those immune responses directed against defined autoantigens. Engineered microbes provide a natural platform for achieving this specificity. By displaying autoantigens on their surface or secreting antigenic peptides in tolerogenic contexts, microbes can drive deletion, anergy, or regulatory differentiation of autoreactive T cell clones. This approach has direct relevance for diseases such as type 1 diabetes, multiple sclerosis, celiac disease, and rheumatoid arthritis, where dominant autoantigens have been well characterized.

The tolerogenic outcome of antigen delivery is critically dependent on the immune context in which antigens are encountered. Engineered microbes exploit this principle by coupling antigen presentation to the simultaneous release of IL-10, TGF- $\beta$ , retinoic acid, or regulatory metabolites [142,143]. This combined signal environment biases antigen-presenting cells toward non-inflammatory phenotypes and drives the expansion of antigen-specific regulatory T cells rather than pathogenic effector populations [145,146]. In this way, engineered microbes recapitulate key features of oral tolerance that are often defective in autoimmune states.

Restoration of the regulatory T cell and Th17 cell balance is another central objective of microbial tolerance engineering [147]. Many autoimmune and inflammatory bowel diseases are characterized by excess Th17-driven inflammation and insufficient regulatory control. Engineered commensal microbes can be programmed to promote regulatory T cell differentiation through multiple complementary mechanisms [63,143]. These include secretion of IL-10, production of short-chain fatty acids, modulation of tryptophan metabolism, and delivery of tolerogenic antigens [148]. By simultaneously suppressing inflammatory differentiation and expanding regulatory networks, microbial platforms act at the level of immune network topology rather than single signaling pathways [63].

Barrier integrity represents a second critical axis of immune tolerance. Breakdown of epithelial barriers allows translocation of microbial products and dietary antigens into underlying tissues, perpetuating chronic inflammation [149]. Engineered microbes can actively participate in barrier repair by stimulating mucus production, enhancing tight junction protein expression, and promoting epithelial regeneration [150]. Some strains can be designed to secrete growth factors, antimicrobial peptides, or mucin-stabilizing enzymes that reinforce the physical separation between luminal contents and immune cells [151]. Restoration of barrier function reduces inflammatory antigen exposure and indirectly supports immune tolerance.

Metabolic regulation forms a further layer through which engineered microbes influence autoimmune immunity. Tryptophan metabolism through the aryl hydrocarbon receptor pathway is

a powerful regulator of mucosal immune balance [61]. Microbial-derived tryptophan metabolites promote regulatory T cell differentiation, suppress pathogenic Th17 responses, and stabilize epithelial homeostasis [152]. Engineered microbes can be programmed to enhance beneficial tryptophan metabolites or reduce pathways that favor inflammation. Similarly, bile acid derivatives modulate innate lymphoid cell activity, T cell differentiation, and macrophage function [153]. By reshaping these metabolic circuits, microbes exert deep control over immune fate decisions.

A defining advantage of microbial tolerance platforms is their capacity for continuous and adaptive immune modulation. Autoimmune diseases typically involve fluctuating inflammatory states driven by environmental, microbial, and metabolic factors. Engineered microbes respond dynamically to these fluctuations by adjusting their regulatory output in proportion to local cues such as cytokines, metabolites, or tissue stress signals [31,63,154]. This adaptive behavior contrasts sharply with static drug dosing regimens and allows tolerance programs to scale with disease activity.

Engineered microbes also offer a route to regional immune specialization. Different autoimmune diseases affect distinct mucosal compartments, including the gut, skin, airway, and urogenital tract [101]. Microbial chassis can be selected or engineered for preferential colonization of these tissues, allowing disease-specific targeting. For example, gut-adapted commensals are particularly suited for inflammatory bowel disease and systemic autoimmunity driven by intestinal dysbiosis, whereas skin-associated strains may be applied to inflammatory dermatoses [59,155,156]. This tissue-specific targeting adds another level of therapeutic precision.

The immune safety profile of microbial tolerance strategies differs fundamentally from that of systemic immunosuppressive drugs [157]. Because regulatory signals are concentrated at mucosal sites and are tightly coupled to local immune inputs, the risk of global immune paralysis is reduced [63,134]. Moreover, microbes can be eliminated through standard antimicrobial strategies if adverse effects arise. This controllability is particularly important for chronic autoimmune conditions that require long-term therapy [104].

Despite these advantages, multiple translational challenges remain. Stability of antigen expression, maintenance of regulatory output under selective pressures, interactions with the native microbiota, and variability between individual host immune environments all influence therapeutic performance [158,159]. Addressing these challenges requires integration of synthetic biology with systems immunology and longitudinal immune profiling in patients [160]. These efforts are essential for moving microbial tolerance engineering from proof-of-concept studies to durable clinical interventions [155].

Taken together, engineered microbes provide a fundamentally new route for re-establishing immune tolerance in autoimmunity and chronic inflammatory disease. Their ability to combine antigen specificity, regulatory cytokine delivery, barrier repair, and metabolic immune control within a single living platform distinguishes them from all existing immunomodulatory technologies. By shifting the therapeutic objective from immune suppression to immune re-education, microbial tolerance engineering opens a new chapter in precision immunotherapy for autoimmune disease.

## 6. Engineered Microbes for Infection Control and Immune Enhancement

Infectious diseases are traditionally approached through antimicrobial strategies that aim to eliminate pathogens directly [22,134]. While these approaches remain essential, their effectiveness is increasingly compromised by antimicrobial resistance, pathogen immune evasion, and host immunopathology. An alternative paradigm is emerging in which engineered microbes are deployed not as antimicrobial agents but as immune enhancers that amplify, reprogram, or coordinate host defense mechanisms [20,161,162]. This immune-forward strategy reframes infection control as a problem of optimizing host immunity rather than solely suppressing microbial growth.

Engineered microbes can be designed to deliver cytokines that selectively amplify anti-pathogen immune responses at sites of infection [163,164]. Localized production of interferons, IL-12, IL-18, or GM-CSF can enhance macrophage microbicidal activity, promote dendritic cell maturation, and

accelerate cytotoxic lymphocyte recruitment [165,166]. Because these cytokines are produced only within infected tissues in response to inflammatory or pathogen-derived inputs, immune activation is spatially constrained. This reduces the risk of systemic cytokine toxicity while maximizing immune pressure on the pathogen.

Targeted enhancement of mucosal immunity represents another major opportunity for microbial immune engineering. Many infections establish initial footholds at barrier surfaces such as the gut, respiratory tract, or urogenital mucosa [162,163,167]. Engineered commensal microbes can reinforce local IgA production, stimulate mucosal dendritic cells, and promote tissue-resident memory T cell formation [168]. By shaping immune readiness at these portals of entry, engineered microbes can raise the threshold for pathogen invasion and reduce the likelihood of systemic dissemination.

Trained immunity offers a further dimension for microbial immune enhancement. Innate immune cells such as monocytes, macrophages, and NK cells can acquire long-lasting functional reprogramming following exposure to defined microbial ligands [44,169]. Engineered microbes can be designed to deliver these ligands in a controlled manner, inducing beneficial epigenetic and metabolic changes that enhance future antimicrobial responses [91,170,171]. Unlike traditional vaccines that act primarily through adaptive immunity, trained immunity-based microbial platforms prime the innate immune system for heightened responsiveness across a broad range of pathogens.

Engineered microbial ligands that engage pattern recognition receptors provide a powerful mechanism for controlling trained immunity. Selective activation of receptors such as NOD2, dectin-1, or Toll-like receptors can bias myeloid cells toward enhanced cytokine production, increased phagocytic capacity, and improved antigen presentation [172–174]. When these signals are delivered repetitively at low levels by colonizing engineered microbes, they can maintain a state of heightened immune vigilance without inducing overt inflammation.

Synergy between engineered microbes and bacteriophages introduces an additional immune-forward strategy for infection control. Phages can selectively reduce pathogenic bacterial populations, while engineered microbes simultaneously stimulate immune clearance of infected cells and residual pathogens [175,176]. This combination allows direct pathogen reduction to be coupled with immune activation, reducing the probability of relapse and suppressing the emergence of resistant strains [177]. Importantly, phage-induced bacterial lysis also releases pathogen-associated molecular patterns that further amplify host immune responses.

Myeloid cell programming is a central target for microbial immune enhancement. Macrophages and dendritic cells orchestrate the balance between tolerance and immunity and determine the quality of downstream adaptive responses [22,110,178–180]. Engineered microbes can deliver cytokines, metabolites, or ligands that bias myeloid differentiation toward pro-inflammatory, antimicrobial phenotypes. This includes promotion of classically activated macrophages, enhancement of antigen presentation capacity, and increased production of reactive oxygen and nitrogen species for microbial killing.

Natural killer cell programming represents a parallel axis of immune enhancement [163]. NK cells play critical roles in early antiviral defense and in controlling intracellular pathogens. Engineered microbes can be designed to deliver IL-15, IL-12, or IL-18 locally, driving NK cell activation, expansion, and cytotoxic function within infected tissues [181–183]. This localized modulation avoids the systemic toxicity associated with cytokine-based NK cell therapies and strengthens early containment of infection before adaptive immunity is fully engaged.

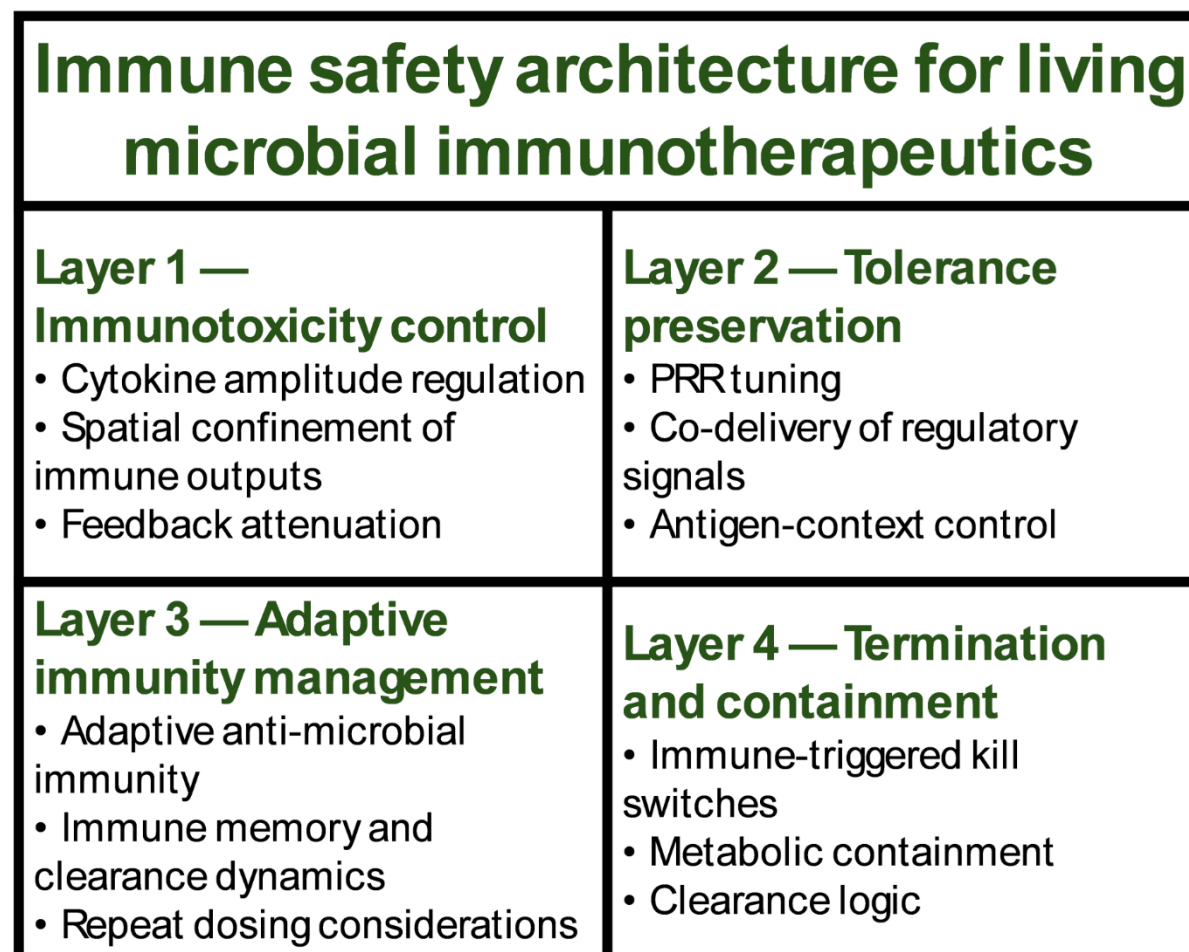
Importantly, immune-forward microbial strategies also address the problem of immune exhaustion that arises during chronic infection [20,84]. Persistent antigen exposure and inhibitory signaling can progressively dampen effector function in T cells and innate populations [184,185]. Engineered microbes can counteract these suppressive circuits by locally delivering co-stimulatory ligands, inhibitory checkpoint decoys, or metabolic modulators that restore immune competence. This capacity to recondition immune responses distinguishes microbial immune enhancement from conventional vaccine or antimicrobial approaches.

The adaptability of engineered microbes further enables context-sensitive immune control across different stages of infection [22,186]. Early in infection, microbes can bias toward immune amplification and effector recruitment. As pathogen burden declines, the same platforms can shift toward resolution programs that promote tissue repair and limit immunopathology. This dynamic regulation reflects the natural progression of host defense and is difficult to reproduce with static pharmacological interventions [4,162,187].

Together, engineered microbes establish a new framework for infection control grounded in immune augmentation rather than direct antimicrobial action. By amplifying innate and adaptive defense mechanisms, inducing trained immunity, coordinating phage-mediated pathogen reduction, and reprogramming myeloid and NK cell function, these systems offer a versatile and resistance-resilient approach to infectious disease therapy. This immune-forward paradigm positions engineered microbes as active architects of host defense rather than passive antimicrobial tools.

## 7. Immune Safety Architecture for Living Immunotherapeutics

The therapeutic power of engineered microbes derives from their ability to engage and reshape immune circuits with high potency, spatial precision, and temporal adaptability [22]. These same properties, however, generate unique safety challenges that differ fundamentally from those associated with conventional biologics, small molecules, or cell therapies. Living immunotherapeutics are capable of sustained replication, autonomous decision-making, and dynamic immune engagement [188,189]. As a result, their safety cannot be reduced to questions of genetic stability or microbial containment alone. Instead, it must be understood through a comprehensive immune safety architecture that governs inflammatory magnitude, tolerance preservation, host-microbe immune memory, and controlled therapeutic termination (**Figure 4**).



**Figure 4. Immune safety architecture for living immunotherapeutics.** The safety of engineered microbial immunotherapies arises from a multi-layered immune safety architecture that extends beyond genetic containment alone. Layer 1 addresses immunotoxicity by regulating inflammatory magnitude through quantitative control of cytokine output, spatial confinement of immune activity, and feedback attenuation to prevent systemic immune escalation. Layer 2 focuses on tolerance preservation, incorporating pattern recognition receptor (PRR) tuning, coordinated delivery of regulatory signals, and antigen-context control to minimize disruption of host immune tolerance. Layer 3 manages adaptive immune responses to the therapeutic microbe, including anti-microbial immunity, immune memory formation, clearance dynamics, and considerations for repeat dosing. Layer 4 defines termination and containment strategies, integrating immune-triggered kill switches, metabolic dependencies, and clearance logic to ensure predictable therapeutic exit. Together, these layers establish immune safety as a systems-level design principle essential for the clinical translation and regulatory evaluation of living immunotherapeutics.

Most existing discussions of microbial safety emphasize the need for genetic kill switches or auxotrophic containment [69,190]. While these measures are essential, they address only one layer of risk. Immune safety operates at a higher systems level and determines whether engineered microbes provoke pathological inflammation, breach tolerance checkpoints, induce deleterious immune memory, or interact unpredictably with host defense programs. The following subsections define immune safety as a multi-dimensional design problem that must be addressed with the same rigor as therapeutic efficacy.

#### 7.1. Immunotoxicity and Unwanted Inflammation

The most immediate and potentially life-threatening risk of microbial immunotherapy is uncontrolled inflammation. Engineered microbes that deliver cytokines, immune ligands, or innate immune agonists are intentionally designed to activate powerful host defense pathways [87]. If this activation exceeds physiological limits or escapes spatial confinement, it can trigger systemic cytokine release, vascular leakage, tissue damage, and organ failure. Unlike conventional drugs, which dilute and decay with predictable pharmacokinetics, living microbes can amplify their own activity through replication and positive feedback within inflammatory microenvironments [116,123]. This creates the possibility of nonlinear immune escalation.

Overshoot inflammation arises when immune outputs exceed the regulatory capacity of local tissue networks. For example, local microbial production of IL-12 or type I interferons may successfully activate cytotoxic immunity within a tumor but can also propagate inflammatory cascades through dendritic cell migration and systemic T cell activation. Similarly, STING agonist delivery that is beneficial within a tumor can induce widespread interferonopathy if signal diffusion is not constrained [123,191,192]. These dynamics make quantitative control of immune output an essential design requirement rather than a secondary optimization step.

Avoidance of systemic cytokine spillover represents one of the core objectives of immune safety engineering. Microbial immunotherapies achieve therapeutic selectivity by exploiting spatial confinement, but spatial control is not absolute. Cytokines and chemokines produced locally can enter circulation, particularly in tissues with high vascular permeability such as tumors or inflamed mucosa [116]. Engineered control strategies must therefore integrate both spatial and quantitative regulation. These include promoter architectures that scale output with local signal intensity, feedback circuits that attenuate production once defined thresholds are reached, and signal-dependent degradation systems that terminate cytokine synthesis in the absence of sustained pathological cues [118,193].

Another critical dimension of immunotoxicity involves the interaction between engineered microbial outputs and endogenous inflammatory loops. Many immune pathways exhibit strong positive feedback, particularly those involving interferons, NF- $\kappa$ B signaling, and inflammasome activation [181,194]. When microbial outputs engage these loops, they can convert localized immune activation into self-propagating systemic inflammation. Effective immune safety architecture must

therefore explicitly model and constrain feedback interactions between engineered signals and host immune amplification circuits [22].

Tissue-specific vulnerability further complicates the inflammatory safety landscape. The gut, lung, and central nervous system exhibit markedly different thresholds for inflammatory injury. Engineered microbes that are well tolerated in the intestinal lumen may be catastrophic if translocated into systemic circulation or ectopic tissues [195,196]. Similarly, mucosal cytokine exposure that supports tolerance can provoke pathology if redirected toward deeper immune compartments [28]. This necessitates tissue-aware immune safety design that accounts for regional immune thresholds, vascular permeability, and resident immune cell composition.

Importantly, immunotoxicity is not limited to acute cytokine release syndromes. Chronic low-grade inflammatory activation can also produce long-term pathology through fibrosis, epithelial barrier erosion, immune exhaustion, and dysregulated tissue remodeling. Engineered microbes designed for long-term colonization must therefore balance sustained therapeutic output against cumulative inflammatory burden [31,156,197]. This balance cannot be achieved through static genetic designs alone and requires dynamic immune-responsive regulation.

Collectively, these considerations establish immunotoxicity as a central and defining constraint in the development of microbial immunotherapeutics. Effective control of overshoot inflammation and prevention of systemic cytokine spillover require integration of spatial targeting, quantitative feedback regulation, tissue-specific immune thresholds, and host-microbe signaling dynamics. Immune safety at this level is not an auxiliary feature but a core architectural principle of living immunotherapy design.

## 7.2. Maintaining Immune Tolerance

A central challenge in the deployment of engineered microbial immunotherapeutics is preserving immune tolerance while deliberately manipulating immune activation. The immune system is organized around layered checkpoints that distinguish harmful from harmless stimuli, and disruption of these checkpoints can result in autoimmunity, chronic inflammation, or tissue-specific immunopathology [128,198,199]. Because engineered microbes operate at the interface of innate sensing, antigen presentation, and metabolic immune regulation, they occupy a position of exceptional influence over tolerance mechanisms. Immune safety therefore demands that tolerance preservation be treated as a primary design constraint rather than an emergent side effect.

Prevention of autoimmunity represents one of the most critical objectives of tolerance-aware microbial engineering. Microbial delivery of immune stimulatory molecules, whether cytokines, ligands, or metabolites, carries the intrinsic risk of lowering activation thresholds in autoreactive lymphocyte populations. This is particularly relevant in genetically predisposed hosts or in tissues with pre-existing low-grade inflammation [56,101,200]. If regulatory networks fail to counterbalance engineered immune activation, autoreactive clones that were previously restrained by peripheral tolerance can expand and initiate sustained tissue damage.

A second major tolerance hazard arises from excessive engagement of pattern recognition receptors (PRR). Toll-like receptors, NOD-like receptors, and cytosolic nucleic acid sensors evolved to detect microbial danger signals with high sensitivity [201,202]. Engineered microbes, even when attenuated, retain molecular structures that engage these receptors. Overactivation of PRR signaling can override regulatory circuits, amplify inflammatory cascades, and drive pathological immune activation independently of antigen specificity. This risk is magnified when microbial load increases or when engineered output reinforces PRR signaling through positive feedback.

Tolerance-preserving microbial design therefore requires quantitative control of PRR activation rather than simple presence or absence of stimulation. This can be achieved by modifying cell wall components, attenuating flagellar or lipopolysaccharide signaling strength, and engineering conditional expression of pro-inflammatory motifs [203,204]. By tuning the magnitude and duration of PRR engagement, engineered microbes can remain immunologically visible without overwhelming tolerance thresholds.

An additional layer of tolerance control involves deliberate co-delivery of regulatory signals alongside immune-stimulatory outputs. Simultaneous secretion of IL-10, TGF- $\beta$ , or regulatory metabolites can buffer the effects of PRR activation and preserve regulatory T cell dominance [63,205]. This dual-output strategy reflects natural host responses to commensal microbes, which continuously stimulate innate sensors while maintaining tolerance through compensatory regulatory signaling [206,207]. Engineered systems that fail to incorporate such counter-regulation risk driving immune imbalance even when individual components appear safe in isolation.

Antigen context further modulates tolerance outcomes. When engineered microbes display antigens, the immune interpretation of those antigens depends entirely on the accompanying signal environment. The same antigen presented in an inflammatory context can provoke pathogenic immunity, whereas presentation in a regulatory context induces tolerance. Immune safety therefore requires that antigen display be explicitly coupled to tolerogenic circuit states and never be allowed to operate under uncontrolled inflammatory conditions [56,198,205].

Host heterogeneity adds another layer of complexity [208,209]. Baseline immune tone varies widely among individuals due to genetics, microbiota composition, metabolic status, prior infections, and age. A microbial design that preserves tolerance in one host may provoke autoimmunity in another. This underscores the need for immune-adaptive circuits that sense host inflammatory state and dynamically adjust output to remain within safe regulatory boundaries.

Taken together, maintaining immune tolerance in the context of microbial immunotherapy is not a passive property but an active engineering problem. It requires simultaneous control of PRR engagement, quantitative regulation of immune outputs, coordinated delivery of regulatory signals, and adaptive responsiveness to host immune state. Tolerance preservation emerges not from microbial attenuation alone but from dynamic systems-level immune design.

### 7.3. Adaptive Immunity Against the Therapeutic Microbe

While innate immune interactions dominate early host responses to engineered microbes, adaptive immunity ultimately determines long-term therapeutic efficacy and safety [210,211]. Host immune systems are highly efficient at recognizing, remembering, and eliminating microbial antigens. Once adaptive immune memory is established against a therapeutic microbe, repeated administrations may fail due to rapid clearance, neutralization of microbial products, or destructive immune responses against colonized tissues. This adaptive barrier represents one of the most underappreciated challenges in living immunotherapy.

Loss of efficacy is often the first observable consequence of anti-microbial adaptive immunity. Antibody-mediated neutralization can block microbial colonization, prevent adherence to mucosal surfaces, and eliminate circulating bacteria before they reach their therapeutic target. Cytotoxic T lymphocytes can recognize and destroy host cells harboring engineered microbes, particularly in intracellular platforms [212–215]. These processes can eliminate the therapeutic agent long before sufficient immune modulation is achieved.

Adaptive immunity also imposes evolutionary pressure on microbial platforms. Once a microbe is recognized as immunogenic, selection favors variants that escape immune recognition. This can destabilize engineered circuits, eliminate control modules, or alter immune outputs in unpredictable ways [104,216,217]. Immune-driven selection therefore introduces a unique form of functional drift that is not present in conventional pharmacological therapies.

Design strategies for evading anti-microbial immunity must operate within ethical and safety boundaries. Complete immune invisibility is neither achievable nor desirable, as controlled immune recognition is essential for safety and clearance [218]. Instead, microbial platforms can be engineered to reduce dominant antigenic epitopes, limit surface exposure of immunogenic structures, or cloak themselves using host-derived molecules [219,220]. Additional approaches include intermittent expression of therapeutic functions, phase-variable surface antigens, or shielding strategies that reduce antibody accessibility.

Predictable immunological memory further complicates repeated dosing regimens [129]. Once immune memory is established, subsequent microbial administrations may provoke faster and more intense inflammatory responses than the initial exposure [221]. In some contexts, this can enhance therapeutic efficacy. In others, it can produce excessive inflammation or rapid microbial elimination [222]. Immune safety architecture must therefore incorporate strategies for managing secondary exposure dynamics rather than focusing exclusively on primary responses.

Microbial platforms that rely on long-term colonization face particularly stringent adaptive immunity challenges. Even commensal-like strains can eventually be targeted when engineered outputs alter immune perception [198,223]. Maintenance of therapeutic populations over extended periods may require immune tolerance induction toward the microbe itself. This creates a complex design tradeoff in which the microbe must remain visible enough for immune clearance if needed, yet tolerated enough to persist therapeutically [224].

Host pre-existing immunity adds another layer of variability. Many chassis organisms belong to species that naturally colonize humans or cause prior infections. Pre-existing antibodies and memory T cells may eliminate engineered derivatives before they can exert any effect [215,223,225]. This variability further supports the need for personalized or stratified microbial immunotherapeutic design.

Ultimately, adaptive immunity is not merely an obstacle to microbial therapy but an integral component of immune safety. It provides long-term clearance, establishes population-level containment, and limits uncontrolled microbial persistence. Effective immune safety architecture must therefore balance immune evasion for short-term efficacy with immune recognition for long-term safety, treating adaptive immunity as a controlled feature rather than an external threat.

#### *7.4. Kill Switches, Containment, and Immune-Clearance Logic*

Genetic kill switches and containment systems are often presented as the primary safety solution for engineered microbes [103]. While essential, these mechanisms represent only the mechanical layer of a broader immune safety architecture. True safety emerges not simply from microbial self-destruction but from coordinated interaction between engineered termination logic and host immune clearance pathways [128,226]. Living immunotherapeutics must be designed to exit the host environment in a predictable, controllable, and immunologically benign manner [104].

Immune-dependent kill switches represent a powerful safety strategy that directly integrates microbial survival with host immune state. These systems activate microbial self-destruction in response to defined immune signals such as interferons, inflammatory cytokines, or antibody binding. In this configuration, the immune system itself becomes the trigger for therapeutic termination [26,93,227]. As inflammatory cues dissipate or immune memory develops, microbial populations are selectively eliminated, creating a dynamic self-limiting loop.

Environmental containment provides an orthogonal layer of safety [128]. Engineered microbes can be made dependent on synthetic nutrients, non-natural amino acids, or defined metabolic substrates that are absent outside controlled environments [228,229]. This prevents long-term survival outside the host and limits environmental dissemination. In gastrointestinal applications, containment can be achieved by restricting survival to specific pH ranges, bile acid profiles, or nutrient niches that exist only within targeted mucosal compartments [230].

Biosafety levels must be explicitly integrated into chassis selection and design logic. Commensal organisms with long histories of safe human exposure offer intrinsically lower biosafety risk than attenuated pathogens [136,188]. However, even commensal chassis can become hazardous when armed with immune stimulatory circuits. Biosafety classification therefore depends not only on the species but on the immune outputs encoded within the engineered genome [69]. Immune potency, not microbial taxonomy, becomes the dominant determinant of biosafety category.

Clearance logic also governs how microbes are removed when therapy is complete. Passive clearance through immune recognition, active clearance through kill switches, and pharmacological clearance through antimicrobial sensitivity must be coordinated to avoid incomplete elimination

[104,128]. Incomplete clearance can promote chronic immune stimulation, biofilm persistence, or selection of escape variants [226,231]. Conversely, overly aggressive clearance can terminate therapy prematurely and provoke collateral inflammation.

An important but underexplored dimension of kill switch architecture is how microbial death itself influences immunity. Lysis releases pathogen-associated molecular patterns, DNA, and intracellular contents that can trigger secondary inflammatory responses [232]. Kill switches must therefore be designed to avoid synchronized mass lysis that could resemble septic shock at the molecular level [233]. Controlled, staggered, or apoptotic-like microbial death programs represent safer alternatives [53].

Horizontal gene transfer further complicates containment. Engineered immune circuits must be insulated from transfer to native microbiota or environmental microbes [234]. Genetic safeguards such as split circuits, non-canonical codon usage, and self-deleting payloads reduce the probability of functional transfer [104,235,236]. This containment layer is essential for preventing ecological propagation of immune-modulatory functions beyond the intended host.

In sum, kill switches, containment systems, and clearance logic form the mechanical backbone of microbial immune safety. However, their effectiveness depends on seamless integration with host immune recognition, adaptive memory, tissue-specific clearance pathways, and ecological containment. Safety in living immunotherapy is not achieved by a single genetic trigger but by a coordinated termination architecture embedded within host–microbe immune interactions.

## 8. Clinical Translation Landscape Focused on Immune Endpoints

Clinical translation in immunotherapy has undergone a fundamental shift from symptom-based and imaging-based outcome measures toward immune-state-resolved evaluation. Across oncology, autoimmunity, metabolic disease, and inflammatory disorders, therapeutic success is now increasingly defined by the capacity to reprogram immune network structure rather than by short-term suppression of isolated disease markers [128,165,169,237]. This transformation reflects a growing recognition that durable clinical benefit emerges only when immune system organization, regulatory balance, and inflammatory topology are stably reshaped. Representative immune-state-resolved endpoints, safety readouts, and translational implications across cancer, autoimmunity, infection, and cross-indication applications (Table 2).

**Table 2.** Clinical translation of engineered microbial immunotherapies through immune-state endpoints.

Disease area	Immune objective	Immune endpoints measured	Safety readouts	Translational implication
Cancer	Immune activation	Cytokine topology; intratumoral T cell infiltration; antigen spreading	Cytokine release syndrome (CRS) markers; systemic cytokine levels	Defines efficacy through immune remodeling rather than tumor burden alone

Cancer	Reversal of immune suppression	STING activation; dendritic cell maturation; myeloid reprogramming	Local versus systemic inflammation; vascular leakage	Enables spatially confined immune activation with reduced systemic toxicity
Autoimmunity	Immune tolerance restoration	Treg frequency; Treg/Th17 balance; tolerogenic dendritic cell markers	Barrier integrity; absence of systemic immunosuppression	Supports antigen-specific and tissue-restricted tolerance induction
Autoimmunity	Suppression of chronic inflammation	Pro-inflammatory cytokine reduction; regulatory cytokine dominance	Infection susceptibility; immune competence markers	Demonstrates immune re-education rather than broad immune suppression
Chronic inflammatory disease	Barrier repair and homeostasis	Epithelial integrity; mucus production; IgA responses	Microbial translocation; systemic inflammation	Links mucosal immune architecture to clinical benefit
Infection	Immune enhancement	Interferon responses; myeloid activation; NK cell activity	Inflammatory overshoot; tissue damage markers	Enables immune-forward infection control without reliance on antimicrobials
Infection	Trained immunity induction	Epigenetic and metabolic markers of innate memory	Chronic inflammation markers; immune exhaustion	Supports durable, broad-spectrum host defense

Cross-indication	Durable immune remodeling	Immune state stability over time; memory formation	Long-term inflammatory sequelae	Shifts evaluation from transient exposure to sustained immune state change
Cross-indication	Therapeutic controllability	Immune-dependent clearance; reversibility of immune effects	Kill-switch activation; microbial clearance kinetics	Aligns immune dynamics with regulatory expectations for safety and control

In cancer trials, immune endpoints now occupy a central position in response assessment. Longitudinal profiling of cytotoxic T cell activation, interferon signaling, antigen presentation capacity, and myeloid suppressor cell dynamics consistently outperforms radiographic measurements in predicting durable remission [92,104]. Responders typically exhibit coordinated elevation of CD8-positive effector function, restoration of dendritic cell cross-presentation, and suppression of TGF- $\beta$ -driven immune exclusion [238–240]. These immune signatures often precede measurable tumor regression and persist as indicators of long-term immune control.

Cytokine landscapes have emerged as unifying translational metrics across therapeutic platforms. Independent of whether the intervention involves checkpoint inhibition, cell therapy, microbial therapeutics, or gene modulation, successful clinical responses consistently align with specific shifts in cytokine topology [241–243]. Activation of interferon networks, controlled induction of IL-12 signaling, normalization of GM-CSF gradients, and suppression of chronic IL-6 and TNF inflammatory loops recur across effective trials. These cytokine reorganizations represent conserved immune attractor states associated with disease control [244–246].

In inflammatory bowel disease, the clinical meaning of immune tolerance reconstruction has become increasingly precise. Trials now routinely quantify regulatory T cell expansion, FOXP3 expression stability, and restoration of Th17 to regulatory T cell ratios as core efficacy endpoints [123,136,247]. Durable mucosal healing correlates more closely with reestablishment of immune regulatory dominance than with transient reductions in inflammatory cytokines alone [248,249]. This immune-centered interpretation has clarified why many anti-inflammatory drugs achieve short-term symptom relief without inducing lasting remission.

Stratification of patients according to microbiome engraftment status has emerged as a critical immune determinant of therapeutic response. In both autoimmune and oncologic studies, responders frequently demonstrate stable incorporation of therapeutic microbial strains, accompanied by downstream expansion of short-chain fatty acid-producing taxa and tolerogenic dendritic cell phenotypes [92,244,250–252]. Non-responders often display persistent immune rejection of administered microbes driven by unresolved type 1 inflammatory tone, preventing establishment of functional microbial-immune circuits.

Metabolic disease trials have similarly converged on immune inflammation as the dominant axis of clinical translation. Improvements in insulin sensitivity, lipid handling, and hepatic steatosis increasingly correlate with attenuation of macrophage-driven adipose inflammation, suppression of IL-1 $\beta$  signaling, and restoration of anti-inflammatory adipokine profiles [253–255]. These immune shifts frequently precede and predict metabolic normalization more accurately than changes in body

mass or caloric intake alone. This has reframed metabolic disease as an immunometabolic disorder governed by inflammatory network stability.

Clinical safety assessment has likewise transitioned toward immune-centered surveillance. Systemic inflammatory markers such as C-reactive protein, ferritin, IL-6, interferon gamma, and neutrophil-to-lymphocyte ratios now serve as early indicators of therapeutic risk long before overt organ toxicity develops [256,257]. Expanded immune cell panel profiling further identifies shifts in activation thresholds, exhaustion states, and regulatory capacity that forecast adverse immune events. Immune monitoring has thus become the primary early warning system for clinical risk [258,259].

Immune correlates of response now function as the translational bridge between preclinical models and human disease. Animal systems that replicate human cytokine architecture, myeloid polarization states, and T cell differentiation dynamics exhibit markedly higher predictive value than those that mimic phenotypic disease features alone [260–262]. Translational fidelity is increasingly defined by immune-state equivalence rather than by anatomical or histological resemblance.

In the context of engineered microbial therapeutics, immune readouts define the core clinical logic. Therapeutic success is determined by coordinated shifts in innate immune training, antigen presentation efficiency, regulatory macrophage polarization, and systemic cytokine normalization. The engineered organism itself is no longer interpreted as the therapeutic endpoint [56,68,263]. The immune network that it reconstructs represents the true object of clinical intervention.

Neuroinflammatory and neuroimmune trials increasingly apply the same immune-centric translational logic. Microglial activation states, peripheral cytokine gradients, and blood–brain barrier immune signaling now serve as primary response metrics in neurodegenerative disease, depression, and chronic pain syndromes. Clinical improvement consistently aligns with normalization of microglial inflammatory tone and astrocytic metabolic coupling rather than with isolated neurotransmitter changes [264–266].

Across cancer, autoimmunity, metabolic disease, and neuroinflammation, a unifying principle has emerged in clinical translation [267–269]. Durable therapeutic benefit correlates with multi-axis immune network reorganization rather than with suppression of single effector pathways. Successful therapies reshape innate training programs, adaptive polarization states, metabolic immune coupling, and regulatory feedback architecture simultaneously. Short-lived responses arise when this coordinated immune restructuring fails to occur.

This immune-first framework also resolves many historical failures in clinical development. Numerous trials that achieved impressive short-term biomarker suppression but failed to induce durable remission now appear mechanistically incomplete. Without stabilization of regulatory balance, inflammatory containment, and immune memory architecture, long-term therapeutic success becomes statistically improbable. Immune architecture, not single target inhibition, governs clinical durability [270,271].

The central translational conclusion is therefore unavoidable. Modern therapeutic success is no longer explained by molecular targeting alone. It is explained by the capacity of an intervention to re-encode immune system state across tissues, time, and disease domains. Immune phenotyping has become the primary explanatory framework of clinical translation and the dominant lens through which the future of living immunotherapeutics must be interpreted.

## 9. Future Directions Toward Personalized and AI-Designed Immune Microbial Therapies

The future of engineered microbial immunotherapy lies not in incremental optimization of existing platforms but in the emergence of a fully integrated design ecosystem that unites systems immunology, artificial intelligence, and programmable biology. As immune phenotyping, spatial biology, and computational modeling converge, microbial therapeutics are poised to transition from generalized immune modulators to precisely individualized immune reprogramming agents. This

transformation reflects a broader shift in medicine from population-averaged intervention toward dynamic, patient-specific immune state engineering.

Single-cell and spatial multi-omics technologies now permit direct measurement of immune cell states at unprecedented resolution across tissues and disease stages. These datasets reveal not only which immune cells are present, but how their transcriptional programs, metabolic states, ligand–receptor interactions, and spatial organization evolve during health and disease. In the future, microbial immunotherapies will be designed not to target abstract disease categories but to drive patient immune systems toward defined target immune attractor states identified through these high-dimensional maps.

Spatial immunology adds a critical architectural layer to this personalization paradigm. Immune function is not uniform across tissues but emerges from the geometric arrangement of immune cells, stromal compartments, vascular interfaces, and microbial niches. Engineered microbes will increasingly be designed to engage specific spatial immune microdomains such as perivascular niches, epithelial interfaces, lymphoid aggregates, or tumor margins. Spatially targeted immune reprogramming will replace uniform immune stimulation as the dominant therapeutic logic.

Artificial intelligence and machine learning will become essential design engines for microbial immunotherapy. The combinatorial complexity of microbial chassis selection, sensing module integration, circuit topology, and immune output tuning exceeds what can be explored through human intuition alone. Machine learning models trained on immune multi-omics and microbial genotype–phenotype relationships will be used to predict optimal chassis–circuit–output combinations for specific immune phenotypes. This will shift microbial design from artisanal genetic engineering toward algorithmically guided therapeutic construction.

Digital twins of the immune–microbe ecosystem represent a particularly powerful future development. These computational replicas of patient immune systems, continuously updated with clinical immune profiling data, will allow *in silico* testing of engineered microbial interventions before physical administration. Digital twins will simulate how microbial circuits interact with host immune networks, predict inflammatory risk, estimate durability of tolerance induction, and forecast interactions with concurrent therapies. This will radically compress development cycles and reduce clinical uncertainty.

Designer microbial consortia will increasingly replace single-strain therapeutics. Complex immune disorders rarely arise from defects in a single pathway and instead reflect network-level dysregulation across innate, adaptive, metabolic, and stromal axes. Microbial communities engineered with division of labor, coordinated quorum logic, and layered immune outputs will allow distributed immune control across multiple biological dimensions simultaneously. These systems will function as living immune control networks rather than as isolated molecular effectors.

Organoid and organ-on-chip immune model systems will serve as the experimental substrates for this next generation of microbial design. Human intestinal, hepatic, pulmonary, and tumor organoids integrated with immune cells and microbiota will allow direct measurement of microbial immune reprogramming in physiologically realistic contexts. These platforms will bridge the gap between animal models and human immune architecture, enabling iterative refinement of microbial circuits under clinically relevant conditions.

Integration of microbial immunotherapies with cell-based immunotherapies will define a major convergence trajectory. Engineered microbes will increasingly be used to precondition tissue immune landscapes prior to adoptive T cell transfer, CAR-T therapy, or natural killer cell infusion. By modulating antigen presentation, suppressive myeloid populations, and metabolic barriers, microbes can establish immune niches that enhance the persistence, functionality, and safety of cellular therapies.

A parallel convergence will occur between microbial immunotherapy and next-generation vaccine platforms. Microbes will be designed to act as living adjuvants that continuously shape mucosal and systemic immunity after antigen exposure. Rather than serving solely as antigen

carriers, engineered microbes will define the immunological context within which vaccine-induced memory forms, influencing durability, breadth, and tissue distribution of protective immunity.

The regulatory science of living immunotherapeutics will likewise evolve toward immune-state-based approval frameworks. Instead of relying solely on classical toxicity and pharmacokinetic metrics, future regulatory evaluation will incorporate immune network remodeling, tolerance stability, trained immunity induction, and memory architecture as formal safety and efficacy endpoints. Regulatory definitions of therapeutic mechanism will increasingly reflect immune systems behavior rather than molecular target engagement.

Ethical and societal considerations will become inseparable from technical progress. As microbial immunotherapies acquire the capacity for long-term immune reprogramming and potential heritable ecological effects within the microbiome, questions of reversibility, intergenerational immune influence, and ecological containment will intensify. Immune safety architecture, digital surveillance, and transparent governance will be required to preserve public trust in these living technologies.

The dominant paradigm emerging from these converging trends is clear. Future microbial immunotherapies will no longer be standardized biological products administered uniformly across patient populations. They will be algorithmically designed, immune-state-resolved, spatially targeted, dynamically regulated, and continuously monitored living systems. They will function as programmable extensions of the immune system itself rather than as external pharmacological tools.

In this forthcoming therapeutic landscape, disease will be interpreted not as a static molecular abnormality but as a dynamic immune network configuration that can be measured, modeled, and re-encoded. Engineered microbes will serve as the living interface through which this immune recoding is executed. The era of generic immune modulation is therefore giving way to the era of personalized immune system programming through living microbial computation.

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