Feature Review

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*Special Issue: Manifesting Synthetic Biology*

# Synthetic immunology: modulating the human immune system

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**Humans have manipulated the immune system to dampen or boost the immune response for thousands of years. As our understanding of fundamental immunology and biotechnological methodology accumulates, we can capitalize on this combined knowledge to engineer biological devices with the aim of rationally manipulating the immune response. We address therapeutic approaches based on the principles of synthetic immunology that either ameliorate disorders of the immune system by interfering with the immune response, or improve diverse pathogenic conditions by exploiting immune cell effector functions. We specifically highlight synthetic proteins investigated in preclinical and clinical trials, summarize studies that have used engineered immune cells, and finish with a discussion of possible future therapeutic concepts.**

## Synthetic immunology: at the crossroads of immunology and synthetic biology

Synthetic immunology is an emerging scientific field at the interface of immunology and synthetic biology that employs biological devices to rationally modulate and manipulate immune responses for the benefit of patients. Whereas a correction of the immune response is desired in disorders of the immune system, for example, inhibition of pro-inflammatory mediators in rheumatoid arthritis (RA), immune cells can also be utilized to ameliorate unrelated diseases, for example, by capitalizing on their cytotoxic properties to eradicate tumors. Interference with the immune system can be as simple as administering a recombinant cytokine or as complex as reprogramming immune cells to differentiate into a desired subset at a specific time. Although recombinant cytokines and growth factors or therapeutic antibodies have been given to patients over the past decade or two, many additional promising therapeutics based on the principles of synthetic immunology are currently being tested in preclinical and clinical studies. At the molecular level, antibody derivatives and antibody mimetics have been engineered to improve the safety and efficacy of these novel therapeutics compared with existing biologics. At the cellular level, immune cells have been engineered to change the target of effectors, to improve their cellular functions, or to counteract genetic defects that subvert the cells' function. However, synthetic immunology also embraces the generation of synthetic lymphoid tissue *ex vivo* and the manipulation of the immune response by artificial components, such as nanomaterials, synthetic adjuvants, and vaccines. Indeed, in the future, we may even develop engineered immune cells that remove emboli from the body, or synthetic bacteria that stimulate the resolution of inflammation.

Biotechnological manipulation of the immune response presumes a sound understanding of the nature and therapeutic potential of immune cells (Box 1 and Box 2). The human immune system is a finely tuned and extremely complex network of molecular agents and cells that protects our body from harmful intruders. The success of this defense system in eradicating pathogens without harming our bodies depends on its ability to discriminate between foreign compounds and molecules present within our bodies under physiological conditions. However, because novel therapeutics based on the principles of synthetic immunology are generated from human-derived components, but

### Glossary

**Antibody-dependent cellular cytotoxicity (ADCC):** cells of the innate immune system possess receptors (such as CD16, CD32, and CD64) that bind the $F_c$ region of antibody isotypes IgG1 and IgG3. Crosslinking of these receptors following $F_c$ binding induces activation of the immune cells, resulting in cellular cytotoxicity against cells expressing the respective antigen.
**Biosimilars:** officially approved versions of an innovator biologic, containing the same genetic information as the original.
**Chimeric antigen receptor (CAR):** CARs are synthetic receptors developed to re-target T cells. More specifically, a single polypeptide containing the light and heavy chains of the variable region of IgGs (single-chain variable fragments, or scFvs) is fused to the transmembrane region and intracellular signaling domain of CD3ζ. In the third and newest generation of CARs, multiple signaling domains, such as those of CD28, 4-1BB, and OX40, are combined to augment potency.
**Complement-dependent cytotoxicity (CDC):** the $F_c$ region of IgG1, IgG3, and IgM can bind to the complement factor C1q, activating the complement activation cascade and the membrane attack complex, which ultimate lead to apoptosis of the target cell.
**Cryopyrin-associated periodic syndrome (CAPS):** a family of autoinflammatory syndromes associated with mutations in *NLRP3*, the gene encoding one of the components of the inflammasome, called cryopyrin. Mutations in *NLRP3* lead to increased production of IL-1β.
**Disease-modifying anti-rheumatic drugs (DMARDs):** a group of otherwise unrelated drugs that are defined by their use in RA to slow disease progression.
**Immunogenicity:** the ability of a compound to induce a humoral (antibody-mediated) or cell-mediated immune response.

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**Box 1. Nature and therapeutic potential of innate immune cells**

*Natural killer cells*

Natural killer (NK) cells are able to recognize and kill infected tumor cells through binding of specific NK receptors to stress-related proteins expressed on the surface of cells, or by the interaction of Fc receptors with antibody-labeled target cells. NK cells use two different routes to induce cell death. On the one hand, these cells release cytotoxic granules containing perforin and granzyme; on the other hand, NK cells trigger apoptosis of the target cell by inducing surface expression or release of death-receptor ligands. These cytotoxic capabilities of NK cells are being harnessed by adoptive immunotherapies to eliminate tumor cells.

*Dendritic cells*

Dendritic cells (DCs) are professional antigen-presenting cells that sample molecules in the periphery by endocytosis, process them intracellularly and present the antigens via MHC class II molecules on the cell surface. The interaction of DCs with lymphocytes in the lymph nodes via MHC class II and the TCR or BCR triggers an immune response via the adaptive immune system. The therapeutic use of DCs in cancer is exemplified by sipuleucel-T, which is a cellular product based on enriched antigen-presenting cells that are briefly cultured with a fusion protein of prostatic acid phosphatase and granulocyte–macrophage colony-stimulating factor (GM–CSF) [99], approved by the FDA for the treatment of prostate cancer. Treatment of cancer patients with sipuleucel-T resulted in a 4-month prolongation of the median survival in Phase III trials [100].

*Monocytes/macrophages*

Monocytes, which develop into macrophages upon moving into tissue, are professional phagocytes that clear dying cells and microorganisms. These cells also play an important immunomodulatory role by stimulating or inhibiting diverse immune cells via direct interaction and by producing cytokines, including TNF and IL-1. Monocytes can be fed immunomodulatory microparticles to reduce their accumulation at the site of inflammation [91] or engineered to help in bone regeneration (because the mediators of monocytes are osteotropic) [101].

*Granulocytes*

Granulocytes patrol the circulation for intruders. These cells are activated by the binding of pathogen-derived molecules to so-called pattern recognition receptors that are expressed on the cell surface or within the cytosol. Elimination of microorganisms is achieved by phagocytosis, release of reactive oxygen species, degranulation of toxic mediators and trapping by ejecting DNA [102]. Because of the short half-life of granulocytes and their terminal differentiation status, the genetic manipulation of granulocytes and, therefore, engineering of these cells is challenging [103]. However, myeloid precursors may be reprogrammed to travel toward a synthetic stimulus via chemotaxis [93] during infections.

**Box 2. Nature and therapeutic potential of adoptive immune cells**

*B lymphocytes*

B cells recognize non-self molecules by binding of B cell receptors (BCRs). These molecules are subsequently endocytosed, processed and presented on the cell surface by MHC class II molecules. Hence, B cells are professional antigen-presenting cells. Interaction with T cells via TCR-MHC class II binding results in the activation of B cells and their differentiation into plasma B cells (antibody factory) and memory B cells (antigen memory).

*T lymphocytes*

T cells recognize antigens when presented to the T cell receptor (TCR) by host cells in the context of an MHC molecule. On the one hand, $CD8^+$ T cells are activated by non-self antigens presented by MHC class I molecules and, hence, by cells that are infected with a virus or a bacterium or by tumor cells. This activation of $CD8^+$ T cells results in the release of, not only perforin and granzyme, but also TNF and elimination of the target cell. In addition, apoptosis of the target cell is induced via the FasL–FAS interaction. On the other hand, $CD4^+$ T cells are activated by antigens bound to MHC class II expressed on professional antigen-presenting cells, resulting in the release of cytokines and in modulation of the immune response. Depending on the co-stimulation of $CD4^+$ T cells by cytokines released from the antigen-presenting cells, T cells are stimulated to produce and release different sets of cytokines. The cytotoxic and immunomodulatory properties of T cells can be used in therapeutic settings. Although current approaches primarily engineer T cells to redirect TCR specificity, other diverse applications include the engineering of co-stimulation [53], the prevention of apoptosis [104], and the induction of inflammation [105].

often combined with other components to form nonendogenous proteins, antibodies or T cell receptors (TCRs) may recognize these molecules as non-self and therefore neutralize them and mount an immune response. Hence, the aim of synthetic immunology is to manipulate the immune response to the benefit of patients by using the support of autologous molecules and immune cells in specifically targeting pathogenic proteins and cells without eliciting adverse effects.

In this review, we describe the current biotechnology-based therapeutic approaches that employ biological devices (molecules and cells) to modulate the immune response, not only in patients suffering from immune disorders, but also in patients with cancer or those undergoing organ transplantation. We discuss the challenges posed by manipulation of the immune system and speculate about future treatment strategies. We foresee that the application of synthetic immunology will be an increasingly powerful way to manipulate, stimulate, and control the immune system to counteract many pathological conditions.

## Engineering molecules of the immune system

While a vast number of therapeutic molecules can interfere with the human immune system, in this review, we specifically discuss those molecules that have been engineered based on human-derived components, such as therapeutic antibodies, chimeric proteins, antibody derivatives, and antibody mimetics (Table S1 in the supplementary material online). The first generation of these engineered molecules of endogenous origin includes recombinant human cytokines and growth factors (Box 3). The interested reader is also referred to excellent reviews covering synthetic vaccines [1,2], synthetic adjuvants [3], synthetic nanomaterials [4] and antibody-recruiting molecules [5].

### *Therapeutic antibodies – molecules with multiple functions*

The potential of treating human diseases with therapeutic antibodies was recognized in the middle of the $20^{th}$ century. The first engineered monoclonal antibodies were reported in the 1970s by Milstein and Köhler [5], and the first member of this class, muromonab-CD3 (OKT3), was approved by the Food and Drug Administration (FDA) in 1986 as an antibody marketed for reducing transplant rejection by interfering with T cell activation. Currently, approximately 30 therapeutic antibodies have been approved, and more than 300 compounds are undergoing clinical trials [5]. Therapeutic antibodies may be generated in mice and subsequently engineered to produce more