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Therapeutic Boosting of the Immune Response: Turning to CD14 for Help

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Abstract: The Toll-like family of immune receptors (TLRs) are critical for an efficient immune response to a variety of microorganisms and other antigens that may cause pathology. Modulating immune responses by targeting TLRs therefore has substantial therapeutic potential, and a number of TLR-based therapeutic strategies have been developed. Minimizing the adverse effects that may result from the therapeutic manipulation of these signalling receptors nevertheless remains a major challenge. Efficient responses via TLRs require the activity of the co-receptor CD14, which enhances TLR responses. In an attempt to boost the immune response for therapeutic purposes, we have sought to target CD14 to achieve TLR modulation. Here we discuss the design, activity and therapeutic development options of TLR-derived peptides that interact with CD14 and enhance its co-receptor activity, thus amplifying TLR-mediated responses. This strategy represents a promising alternative to current TLR-based therapies, as it has the potential to amplify responses to different pathogens mediated by different TLRs by targeting the common TLR co-receptor, CD14.

Keywords: Immune response, Toll-like receptors, CD14, Toll-like receptor-based therapies, vaccines, peptides.

1. INTRODUCTION

Enhancing the immune response has vast therapeutic potential, not only for the treatment of immune deficiencies, but also for the development of more efficient vaccines, and the treatment of allergies and cancer. In this context, Toll-like receptors (TLRs) are considered major therapeutic targets, as they play a pivotal role in the body's immune response. TLRs are type I transmembrane proteins expressed primarily on immune competent cells and also in a variety of other cell types. They detect microbial pathogens and trigger pro-inflammatory host defence responses that ultimately clear infections [1, 2]. TLRs also orchestrate adaptive immunity, resulting in efficient responses to viruses, bacteria, parasites, tumours and allergens. Dysregulation of TLR responses may cause pathology. Excessive TLR-mediated responses to microbial pathogens and other antigens can lead to pathological inflammation (e.g. septic shock, arthritis, asthma and autoimmunity). By contrast, TLR hyporesponsiveness may result in susceptibility to, and failure to clear, infections (e.g. sepsis-induced immunosuppression). TLR dysregulation may also affect the quality and/or magnitude of adaptive immune responses, because TLR activation promotes maturation of antigen-presenting cells (APCs) such as dendritic cells (DCs) into fully competent APCs, guiding T cell differentiation toward $CD4^+$ T-helper 1 (T_H1) cells or $CD8^+$ cytotoxic lymphocytes [3-6].

An efficient response to a variety of microbial components and other antigens by most TLRs requires the activity of the TLR co-receptor CD14. This co-receptor amplifies TLR-mediated pro-inflammatory and immunomodulatory responses [7-10]. CD14 is expressed at the cell surface (mainly monocytes, macrophages and neutrophils) and as a soluble co-receptor (sCD14) in plasma and other biological fluids [11, 12]. Co-receptor activity requires interaction of CD14 with the TLR, because CD14 is believed to deliver the microbial component to the TLR for optimal recognition [13].

A number of therapeutic strategies are being developed that use TLR agonists as vaccine adjuvants or monotherapeutics, or use TLR antagonists to reduce pro-inflammatory responses [3, 4, 14]. However, achieving optimal clinical benefits, while minimizing the adverse effects that may result from the therapeutic manipulation of these potent signalling receptors, remains a major challenge. Here, we discuss a promising alternative to current TLR-based therapeutic strategies to boost the immune response that is based on our recent work aimed at amplifying the co-receptor activity of CD14 [15].

2. THERAPEUTIC RATIONALE AND CLINICAL DEVELOPMENT OPTIONS

2.1. Therapeutic Rationale of the CD14-Targeting Strategy

We have sought to target the TLR co-receptor CD14 for therapeutic purposes. We hypothesized that by targeting CD14 and increasing its co-receptor capacity, the immune response can be boosted. This approach raises the possibility of amplifying immune responses to pathogens and other antigens irrespective of their nature and of the TLR(s) involved in their recognition, because the target for modulation is the

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common TLR co-receptor, CD14. Furthermore, this strategy does not require direct manipulation of the TLRs or their signalling pathways.

2.2. Clinical Development Options

2.2.1. Sepsis

A promising and immediate application of the CD14-targeting strategy currently being evaluated in our laboratory is for the treatment of sepsis-induced immunosuppression, the later phase of sepsis with the highest mortality rate. Sepsis is a life-threatening clinical condition characterized by a widespread and harmful inflammation resulting from the immune system's overreaction to severe infection. It can lead to organ dysfunction and failure, resulting in admission to an intensive care unit (ICU). Sepsis is a major healthcare problem, killing over 37,000 people a year in the UK alone – more than lung cancer or bowel, breast and prostate cancer combined. In the USA, sepsis is the 10th leading cause of death, killing over 200,000 people annually. It constitutes a substantial social and financial burden to healthcare systems worldwide, and is increasing in incidence [16]. Sepsis patients typically exhibit a biphasic immune response to infections. Survivors of an initial hyper-immune state, characterized by widespread inflammatory tissue injury, manifest a state of immune hyporesponsiveness, which includes anti-inflammatory responses and TLR hyporesponsiveness to pathogens. These patients are unable to mount an appropriate pro-inflammatory response to clear infecting organisms, and are very susceptible to subsequent infections by opportunistic pathogens [6, 17]. These so-called 'second-hit' infections (e.g. nosocomial infections), have a high mortality rate and, even when not fatal, result in numerous adverse outcomes including prolonged periods of costly organ support (e.g. mechanical ventilation) in ICUs. Thanks to improvements in patient management, the majority of sepsis mortality is no longer due to immune overreaction during the acute phase of the illness, but instead due to immunosuppression. The immunosuppression stage represents a more promising avenue of investigation for an immunomodulatory therapy than the initial hyper-immune phase, which is usually well established before a therapeutic window presents itself. For this reason, and given the failure of numerous strategies aimed at attenuating the initial "cytokine storm", therapies aimed at enhancing immune responsiveness during the immunosuppression phase of the illness are now being evaluated [17, 18]. The CD14-based therapeutic strategy proposed here could prove to be a valuable adjunct to standard antibiotic therapies. It would restore the premorbid responsiveness of immune cells to microorganisms, thus reducing patients' susceptibility to secondary infections. Correct timing for interventions is critical for the use of immunostimulatory therapies in these patients, as both pro- and anti-inflammatory responses do not necessarily represent subsequent events but might, at least in part, occur concomitantly [17, 19]. The extent of the pro- and anti-inflammatory responses depends on many factors, including pathogen virulence, bacterial load, host genetic factors, age and comorbidities [17]. Thus, patient stratification through the identification of those in the immunosuppression phase, for example, using immune status biomarkers (e.g. HLA-DR, IL-10 levels) and/or genetic screening will be of paramount importance for the timely use of immunity-boosting approaches.

2.2.2. Vaccine Development

The therapeutic potential of the proposed CD14-targeting strategy is not limited to the treatment of sepsis. Given the ability of TLRs to promote maturation of DCs into fully competent APCs, TLR agonists are being exploited and evaluated as vaccine adjuvants for infectious diseases, allergies and cancer. By inducing APC maturation, TLR agonists help to improve the presentation of pathogen-, allergen- or tumour-derived antigens, leading to more efficient and specific adaptive and innate immune responses [3, 4, 20]. Efforts are underway to design/discover new TLR agonists with increased efficacy, in order to reduce adjuvant and/or antigen concentration and thus lower possible vaccine toxicity and cost. In this context, using a CD14-enhancing molecule as co-adjuvant to amplify TLR activation would increase the potency of the vaccine, allowing for a reduction in the dose of antigen and/or adjuvant used. Our recently reported work [15] and current studies summarised below (secn. 3.1.) support this claim.

2.2.3. Improved Monotherapies

In addition to their use as vaccine adjuvants, TLR agonists are also being evaluated as monotherapeutics [3, 4]. Agonists for TLR3 and TLR7-TLR9 show significant promise for the treatment of cancer as well as infectious diseases, especially viral infections (e.g. imiquimod/TLR7 agonist, basal cell carcinoma and human papilloma virus infection; polyI:C/TLR3 agonist, $TLR3^+$ breast carcinoma cells; CpG-oligodeoxynucleotides/TLR9 agonist, $TLR9^+$ chronic lymphocytic leukemia cells and hepatitis C). The CD14-targeting molecules could improve the efficacy of TLR-based monotherapeutics by amplifying TLR activation and helping to reduce the agonist's dose and toxicity. CD14-boosting molecules could also serve as valuable adjuncts to existing therapies for other pathologies in which amplifying TLR activity may be beneficial. For example, we are currently testing whether accelerating infection resolution by amplifying CD14's activity, as we reported, could lower the extent or frequency of peritonitis episodes in peritoneal dialysis patients, improving dialysis efficiency.

3. POTENTIAL THERAPEUTIC TOOLS: CURRENT AND FUTURE DEVELOPMENTS

3.1. CD14-Targeting TLR Stimulatory Peptides

By screening a library of peptides representing the leucine-rich repeats (LRR) of the human TLR2 extracellular domain (known to be involved in molecular recognition) and conducting site-directed mutagenesis analysis, we have identified LRR 5, 9, 15 and 20 as those involved in the interaction with the co-receptor CD14 [15]. Peptides corresponding to LRRs 5, 9, 15 and 20 showed an immunostimulatory effect. The most potent of them, peptides 5, 9 and 15 (corresponding to TLR2 LRRs 5, 9 and 15), strongly enhanced neutrophil and monocyte chemoattractant (chemokine) release by leukocytes in response to TLR ligands or whole

bacteria. Consequently, the peptides increased phagocyte recruitment to the peritoneum of the infected mice, and accelerated bacterial clearance in mouse models of Gram-positive (*Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*) bacterial peritonitis [15]. Mechanistically, TLR2-derived peptides specifically interacted with CD14 and accelerated microbial ligand transfer from CD14 to the TLR, resulting in increased and sustained ligand occupancy of TLR. This led to increased TLR clustering for signaling, and thus an amplified response to microbial components (Fig. **1**). Of note, the peptides showed no activating capacity *per* se [15].

Notably, the TLR2-derived peptides 5 and 9 were shown to rescue pro-inflammatory responses of sepsis-induced immunosuppressed patients *ex vivo* [15]. We found that whole blood from patients with severe sepsis, tested immediately after admission to ICUs, was profoundly immunosuppressed. This was indicated by the reduced release of neutrophil and monocyte chemo attractants in response to stimulation with TLR agonists when compared with blood from normal donors. However, in the presence of TLR2 peptide 5 or 9, chemo attractant release by the stimulated blood from patients substantially increased. The degree of enhancement of the patients' response depended on the inflammatory mediator, the microbial component and the patient tested.

Up-modulating the activity of CD14 with TLR2-derived peptides also affected adaptive immune responses [15]. DC maturation following treatment with lipopeptide or *Mycobacterium*-derived purified protein derivative (PPD) was enhanced in the presence of peptide 9, as indicated by the increased expression of major histocompatibility complex class II, CD83 and CD86. Consistent with its positive effect on DC maturation, peptide 9 also markedly enhanced interleukin-2 (IL-2) production by PPD-stimulated peripheral blood mononuclear cells, indicating T cell activation and expansion [15]. Modulating CD14 activity also affected the cytokine profile of DCs upon stimulation [15]. DC activation by a typical microbial product, lipopolysaccharide (LPS), in the presence of peptide 9 resulted in a significant and preferential increase in the release of IL-12 and interferon-γ (IFN-γ), which are crucial mediators of $CD4^+$ T_H1 T cell differentiation and expansion. Overall, modulating the activity of CD14 demonstrated clear potential to affect the extent and quality of the adaptive immune response and favour a strong T_H1 T cell response.

Thus, increasing CD14's co-receptor activity could be exploited for the development of better vaccine adjuvants, by promoting efficient antigen presentation and T cell expansion. Recent unpublished work from our laboratory supports this possibility. Immunisation of mice with the model antigen

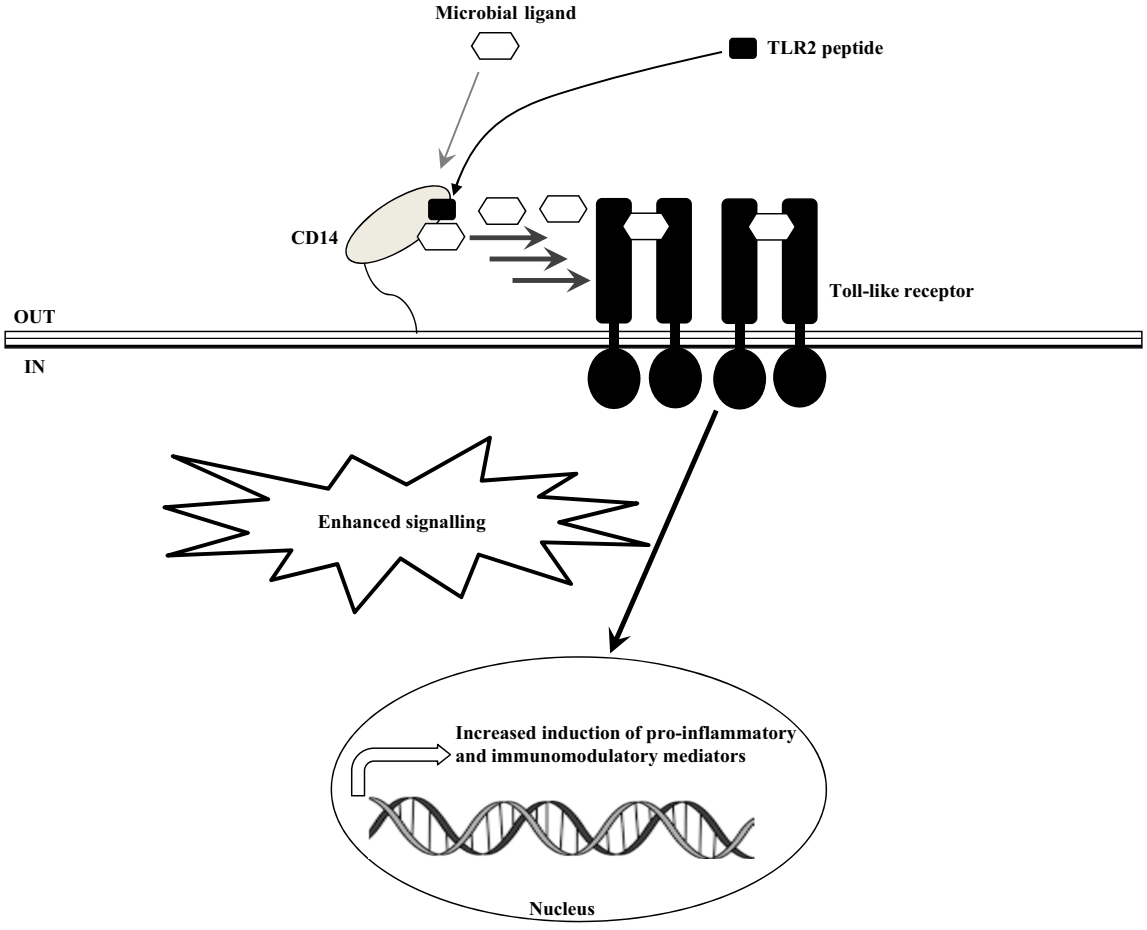

Fig. (1). Schematic diagram illustrating the CD14-targeting strategy to boost TLR activity. TLR2-derived peptides increase CD14's co-receptor activity, and thus enhance TLR responses, by specifically binding to CD14 and accelerating ligand transfer from CD14 to the TLR. This results in increased and sustained ligand occupancy of TLR, increased receptor clustering for signaling, and thus an amplified response.

ovalbumin (OVA) resulted in the production of both anti-OVA-specific plasma immunoglobulins (Igs) IgG1 and IgG2a. Anti-OVA IgG levels increased only slightly (not significantly; IgG1: ~25%, IgG2a: ~17%) when the mice were immunised with OVA in the presence of the TLR2 ligand Pam_3-Cys-Ser-Lys_4 (Pam_3Cys) lipopeptide, used as adjuvant. However, immunization with OVA in combination with Pam_3Cys and the TLR2 peptide 5 resulted in a significantly increased production of both anti-OVA IgG1 and IgG2a, when compared with OVA+Pam_3Cys or OVA alone, with predominance of IgG2a (68% increase *vs.* OVA+ Pam_3Cys) over IgG1 (37% increase *vs*. OVA+ Pam_3Cys). Of note, administration of peptide 5 in combination with OVA in the absence of the adjuvant Pam_3Cys did not affect the levels of anti-OVA Igs. This supports the concept that the TLR2 peptides have no activating capacity *per* se, as we previously reported [15]. Increasing the dose of OVA antigen resulted in significantly higher plasma levels of anti-OVA Igs, both in the absence and presence of the adjuvant Pam_3Cys. Notably, when peptide 5 was administered together with Pam_3Cys and the lower dose of OVA, the production of anti-OVA Igs was increased to levels similar to those obtained with the higher dose of antigen in the absence of peptide. This highlighted the ability of the TLR2-derived peptide to promote a more efficient antigen presentation. These findings support the potential use of a CD14-boosting molecule as co-adjuvant, increasing the potency of vaccines through better antigen presentation, while reducing adjuvant and/or antigen concentration. This would lower the possibility of toxicity and may be particularly useful in the case of vaccine antigens difficult and/or expensive to synthesise.

3.2. Development of Peptide Mimicking Small Molecules

The practicality of a therapeutic strategy based on the use of peptides, like the one described here, is supported by the well-documented advantages of biologics (peptides and proteins) over small molecules. Biologics have much higher target specificity due to their large size, exhibiting much stronger interactions, and thus less promiscuity and side effects in drug targeting, than small-molecules [21]. The identification and development of small molecules capable of mimicking the effect of the peptides, however, merits to be attempted. Small molecule drugs offer better metabolic stability, membrane permeability, solubility and lower production cost. To identify candidate molecules, two approaches to screen small chemical compound libraries are being evaluated in our laboratory: *in silico* (virtual) screening and the use of encoded library technology. Selected molecules should bind to CD14 and increase its co-receptor capacity. Cell-based functional assays will then be conducted to select the molecule(s) with optimal co-receptor enhancing capacity.

CONCLUSION

Here we have discussed the therapeutic potential of a strategy to boost the immune response that is based on the capacity of the co-receptor CD14 to amplify the response of a family of receptors that plays a pivotal role in the immune system, the TLRs. Targeting CD14 with TLR-derived peptides demonstrated the ability to enhance TLR activity and thus the immune response to pathogens and other antigens. Given that CD14 works in concert with most TLRs, modulating its co-receptor activity would be potentially useful as a therapeutic approach against a variety pathological conditions in which different TLRs may be involved, e.g. infections, allergies and cancer. The advantage of using peptides *versus* small molecules to modulate CD14 activity merits further consideration.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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