Optical Control of Cytokine Signaling via Bioinspired, Polymer-Induced Latency

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ABSTRACT: Cytokine signaling is challenging to study and therapeutically exploit as the effects of these proteins are often pleiotropic. A subset of cytokines can, however, achieve signal specificity via association with latency-inducing proteins, which cage the cytokine until disrupted by discreet biological stimuli. Inspired by this precision, here, we describe a strategy for synthetic induction of cytokine latency via modification with photolabile polymers that mimic latency while attached then restore protein activity in response to light, thus controlling the magnitude, duration, and location of cytokine signals. We characterize the high dynamic range of cytokine activity modulation and find that polymer-induced latency, alone, can prolong in vivo circulation and bias receptor subunit binding. We further show that protein derepression can be achieved with a near single-cell resolution and demonstrate the feasibility of transcutaneous photoactivation. Future extensions of this approach could enable multicolor, optical reprogramming of cytokine signaling networks and more precise immunotherapies.

INTRODUCTION

Cytokine signaling is critically important to a variety of physiological processes, including cell and tissue development, aging, disease pathogenesis, and the mounting of effective innate or adaptive immune responses. In addition to serving as signal mediators, these proteins can also act as potent therapies with more than 18 cytokine products currently FDA-approved for the treatment of diseases, including chronic hepatitis, multiple sclerosis, rheumatoid arthritis, chronic kidney disease, degenerative disk disease, and multiple types of cancer. Although cytokines hold great potential as tools to both study and treat human disease, in vivo effects of these proteins are often highly pleiotropic and thus difficult to understand and challenging to control.

One mechanism by which cytokines with diverse effects can transmit tissue- and cell-specific information is via expression in an inactive, or latent, form in which the protein is sterically shielded by another peptide or protein binding partner. Transforming growth factor-β1 (TGF-β1), for example, has recently been shown to noncovalently associate with a latency-associated peptide (LAP), which deshields from TGF-β1 in response to traction forces caused by the binding of αV/β6 or αV/β8 integrins with either cell membrane-bound GARP (glycoprotein A repetitions predominant) or extracellular matrix-bound LTBP-1 (latent transforming growth factor beta-binding protein 1). This stimuli-responsive uncaging of the protein can lead to remarkable specificity; interaction with migratory dendritic cells has been shown to present TGF-β1 to naïve CD8+ T cells, preconditioning them for tissue-resident memory fate. Similarly, the interaction with regulatory T cells (Tregs) and microglia has been found to deshield TGF-β1, thus initiating anti-inflammatory signaling cascades in these cells and other nearby cell types.

Inspired by the ability of reversible shielding to impart cell- and tissue-specificity to cytokines expressed in a latent form, we hypothesized that chemical modification with synthetic macromolecules could impart similar or improved specificity to other cytokines not expressed in a latent state. Photoresponsive linker technologies present a potential, synthetic alternative to latency binding proteins, providing spatiotemporal control over cytokine activation and, additionally, orthogonality to existing shielding/deshielding pairs. Historically, photolabile linkers have been utilized to reversibly immobilize peptides and oligonucleotides onto purification resins; however, more recently, this approach has been adapted in order to reversibly cage small molecules, peptides, and nucleic acids. For example, caged neurotransmitters have been used to study memory formation in the brain, caged peptides complexed with a major histocompatibility complex (MHC) have been

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exploited to study structural reorganization at the immune synapse, and caged single guide RNA (sgRNA) has been utilized to spatially constrain gene editing by CRISPR/Cas9. Extension of this strategy to immune signaling proteins—which can be hundreds of times larger—thus represents a significant and unaddressed challenge.

Here, we describe a strategy whereby cytokines are chemically modified with photolabile polymers that mimic the induction of protein latency while attached to deshield to recover the protein activity in response to monochromatic light exposure. This approach enables both the magnitude and the duration of cytokine signals to be tuned on demand, with high spatial resolution, and can be rapidly adapted to a range of additional cytokine or chemokine proteins. Future extensions of this approach could enable optical reprogramming of cytokine signaling networks and could lead to new immunotherapies that are more tissue-specific and patient-personalized.

**METHODS**

**Materials and Supplies.** Unless otherwise specified, reagents were used as received without further purification. Recombinant human IL-2 (200-02, Peprotech), recombinant human IL-15 (570,308, Biolegend), and recombinant mouse scIL-12 (130-096, Miltenyi Biotec) were used as received without further purification. NH-SulfoCy7 and 5 equiv of a photolabile linker to 5% v/v DMSO.

**Protein Characterization.** Cytokine hydrodynamic size was measured by dynamic light scattering (Wyatt DynaPro Plate Reader III) using 2–4 averages of 10–30 s acquisitions. Electrophoretic mobility was measured via polyacrylamide gel electrophoresis under reducing conditions (50 mM dithiothreitol, Bio-Rad). Protein bands were visualized with a Coomassie G250 stain (Bio-Rad) and imaged using a Licor CLx gel imager.

**Cytokine-Induced Proliferation.** Murine CTLL-2 T cells (ATCC) were maintained in RPMI 1640 with high glucose, antibiotics, within 96-well plates and treated with equimolar amounts of wild-type or latent protein for 48 h. After 24 h, the cell culture supernatant was harvested and tested for IFNγ secretion via ELISA (R&D Systems). Optical density at 450 nm was measured using a Spectramax i3 plate reader (Molecular Devices), and results were compared with standard curves. These studies were approved by Emory University’s Institutional Animal Care and Use Committee.

**JAK/STAT Pathway Activation.** STATT-SEAP reporter cells (HEK293, Invivogen) were maintained in DMEM supplemented with 4.5 g/L of glucose, 2–4 μM of latent IL-2 (1000 IU/mL or equimolar amounts modified protein for 48 h. Then, 20 μL of cell culture supernatant was withdrawn and analyzed for the alkaline phosphatase content via change in QUANTI-Blue (Invivogen, rep-qs) absorbance at 620 nm (Spectramax i3 plate reader).

**Binding Affinity.** Cytokine and cognate receptor binding kinetics were measured via biolayer interferometry using an Octet RED344 system (FortecBio). Nickel nitrotriacetic acid sensors (Ni–NTA, FortecBio) were equilibrated in PBS and coated with polyhistidine-tagged receptor proteins (SinoBiological) for 5 min (1.5 μg/mL IL-2Rα, 3.3 μg/mL IL-2Rβ). Association kinetics were measured over 5 min at 20 μM IL-2 followed by dissociation in PBS over 5 min. Measurements were repeated in three independent experiments and fit using Data Analysis 8.0 (FortecBio) using a 1:1 kinetic model.

**Pharmacokinetics.** C57BL/6 mice (female, 7 weeks) were injected via the tail vein with 2 μg scIL-12 protein conjugated with Cy7 alone or Cy7 with photolabile poly(ethylene glycol). Plasma was collected from 100 μL of blood obtained via submandibular bled into heparinized tubes (BD Microtainer). Plasma fluorescence was integrated using Image) software after polyacrylamide gel electrophoresis. Mice with peak plasma fluorescence <1.2x above the baseline were excluded. These studies were approved by Emory University’s Institutional Animal Care and Use Committee.
Figure 1. Bioinspired cytokine latency via photolabile polymer modification. (a) Transforming growth factor-β1 (TGF-β1) transmits tissue- and cell-specific cytokine signals via association with a latency-associated peptide (LAP), which sterically shields and later disassociates from TGF-β1 in response to traction forces caused by the binding of αVβ6 or αVβ8 integrins with either cell membrane-bound GARP or extracellular matrix-bound LTBP-1. (b) Strategy for the induction of reversible latency in cytokines with pleiotropic effects via modification with end-modified, photolabile polymers. (c) Illustration of the traceless modification strategy used here whereby 20 kDa poly(ethylene glycol) polymer chains are appended to cytokine lysine residues via o-nitrobenzyl groups, which are (d) rapidly degraded by blue, but not green, LED light as measured by cleavage-induced fluorescence dequenching. Data in panel (c) represent mean ± SD of three technical replicates.

Figure 2. Polymer-induced latency augments cytokine size, biases receptor subunit binding affinity, and prolongs in vivo circulation. (a) Stepwise increase in IL-2 hydrodynamic size upon linker and polymer conjugation as measured by dynamic light scattering [diameter in nm, (Polydispersity index): 11.5 (0.1), 17.1 (0.1), 38.5 (0.2)]. (b) Sensograms depicting binding kinetics for IL-2 or latent IL-2 association/dissociation with IL-2Rα (CD25) or IL-2Rβ (CD122). (Inset) Fold change in postmodification binding affinity. (c) Electrophoretic mobility shift demonstrating Cy7 dye- and polymer-dependent modification of scIL-12 as measured by polyacrylamide gel electrophoresis. (d) Increase in scIL-12 hydrodynamic size following Cy7 conjugation with or without linker/polymer modification as measured by dynamic light scattering. (e) Plasma pharmacokinetics of Cy7-labeled scIL-12 modified with or without a linker/polymer following intravenous injection into C57BL/6 mice, illustrating prolonged circulation following photolabile polymer modification. Data in panel (b) represent mean ± SD of three technical replicates. Data in panel (e) represent mean ± SEM of two to three biological replicates. Curve fits in panel (e) were constrained to decay to average fluorescence values from vehicle-treated mice. Error bars in panel (e) smaller than data point sizes are obscured. *p < .05, **p < .01, ***p < .001, ****p < .0001.
To demonstrate polymer-induced cytokine latency, we selected recombinant human IL-2 as a candidate for photolabile polymer modification due to its lack of a known latency binding partner and its well-described pleiotropic effects in vivo, for example, its simultaneous immunostimulatory effects exerted via cytotoxic T cells and immunosuppressive effects exerted through regulatory T cells. We also selected IL-2 as the protein that has demonstrated clinical benefits in patients with melanoma, renal cell cancer, and neuroblastoma. These benefits are greatly limited by the small size and rapid excretion of IL-2, which necessitates continuous or frequent high-dosing and thus toxicity and complex treatment management.21,22 We hypothesized that polymer-induced IL-2 latency could be used to both control IL-2 signaling ex vivo and improve the safety or therapeutic potential of this and related cytokines via prolonged circulation.

Following modification of IL-2, we observed a stepwise increase in size upon both linker and polymer conjugation as measured by both polyacrylamide gel electrophoresis and dynamic light scattering (Figure 2a). Latent IL-2 was approximately threefold larger than the wild-type protein in overall diameter, thus well above the lower size limit for renal clearance in humans. We further examined the binding affinity of latent IL-2 with two of its cognate receptor subunits, IL-2Ra (CD25, rhIL-2 (7 M) and IL-2Rb (CD122, rhIL-2—10−8 M) via biolayer interferometry (Figure 2b). Strikingly, binding affinity of IL-2 toward IL-2Rα was decreased approximately 53-fold following latency induction (20 ± 7 μM), whereas affinity toward IL-2Rβ was nominally lower but failed to reach statistical significance (46 ± 40 μM). This serendipitous result suggests that, relative to wild-type IL-2, latent IL-2 maintains a biased activity toward CD8+ T cells that express IL-2Rα and dampened activity towards immunosuppressive Tregs that constitutively express IL-2Rβ/β.24

Given that the off-target activity toward Tregs is believed to contribute, in part, to the failure of IL-2 therapy in patients, these findings warrant future investigation in mouse models of cancer and other diseases reliant on T cell immune evasion.

Having demonstrated that polymer-induced latency can modulate cognate receptor binding affinity, we characterized the effect of polymer-induced latency on in vivo cytokine circulation using IL-12, another recombinant cytokine under clinical investigation, which similarly suffers from rapid clearance and systemic, off-target toxic effects.26 As therapeutic cytokines are generally quite small (ca. 12–70 kDa), polymer modification—frequently, with PEG—is often used to decrease renal clearance, thus prolonging circulation and augmenting tissue exposure with drugs (e.g., pegfilgrastim, peginterferon, etc.).27 To monitor cytokine circulation in vivo, we dye-labeled a novel single-chain variant of the cytokine, scIL-12, both with and without photolabile polymer modification (20 kDa PEG, Figure 2c). Cy7 labeling only nominally increased scIL-12 size as measured by polyacrylamide gel electrophoresis and dynamic light scattering, whereas combined dye and photolabile polymer modification increased the hydrodynamic size to 44 nm (Figure 2d), well above the renal clearance size threshold in humans and rodents. We then monitored the circulation of latent scIL-12 following tail vein injection in C57BL/6 mice, finding that the latent cytokine experienced an 11-fold increase in the circulation half-life following polymer-modification (Figure 2e). While we observed no large protein aggregates in DLS or electrophoretic mobility measurements of both latent cytokines, we do note that the formation of discreet protein multimers is commonly
observed among clinically approved cytokine therapies, both wild type and PEGylated, and that we cannot exclude the possibility of dye- or polymer-induced multimer formation here, which might affect associated activity or abundance measurements. Physical state notwithstanding, these results suggest that the prolonged circulation of scIL-12 may (i) obviate the need for frequent, high dosing and (ii) improve tissue accumulation of the drug in therapeutic settings.

**Photoactivation of Latent Cytokines.** After characterizing the effects of polymer-induced cytokine latency, we next examined the recovery of functional protein activity using CTLL-2 T cells that depend on both IL-2 and IL-2Rα for growth. Following latency-induction, we observed an approximate 10^3-fold drop in rhIL-2 activity as measured by CTLL-2 T cell proliferation (24 h). Effect of latent IL-2 and uncaged IL-2 (1000 IU/mL molar equivalents) on (c) OVA257−264 antigen-specific T cell activation and (d) JAK/STAT pathway activation as measured ex vivo by ELISA of OT-I splenocyte-secreted interferon gamma (IFNγ, 24 h) and STAT5 reporter cell secretion of alkaline phosphatase (48 h), respectively. Chromogenic substrate absorption in panel (d) was monitored at 620 nm. Data in panels (b) and (d) represent mean ± SD of of 6 technical replicates. p < .01 (**), p < .0001 (****).
and power densities (400 nm, 300 mW/cm², respectively). In vivo, as little as a 10-fold change in rhIL-2 activity is necessary in order to functionally modulate fate decisions in T cells that lead to either the memory or effector fate, thus the near three logs of dynamic range observed here in vitro suggest that latent cytokines may be used to control T cell biology ex vivo or modulate therapeutic activity of the recombinant protein. To further demonstrate the feasibility of this approach, we also examined latency induction with rhIL-15, finding that small molecule linker addition, alone, was sufficient to achieve 5- to 20-fold modulation of CTLL-2 T cell dose-dependent proliferation (24 h, Figure S4).

To further explore the therapeutic potential of latent IL-2, we investigated its ability to promote antigen-specific immunity using OT-1 T cell receptor transgenic mice, which generate clonal CD8⁺ T cells specific to SIINFEKL, an octamer peptide from ovalbumin (OVA257−264). We pulsed OT-1 splenocytes with OVA257−264 and treated with either wild-type or latent IL-2, with or without LED irradiation, and measured IFNγ as an indication of the extent of T cell activation. Although latent IL-2 had no significant effect on antigen-specific T cell activation, that from the light-uncaged protein was comparable and statistically indistinguishable from wild-type IL-2 (Figure 3c).

To ascertain whether the activity of latent IL-2 on OT-1 T cells was, like the wild-type protein, JAK/STAT pathway dependent, we examined its effect on HEK293 cells engineered to express all three subunits of human IL-2R, JAK3, and STAT5. In response to STAT5 activation, these cells secrete alkaline phosphatase, which can be spectrophotometrically detected using a chromogenic substrate. The trends in the reporter cell response observed in these studies closely match those observed in activated OT-I T cells. Latent IL-2 induced near-baseline levels of STAT5 transcriptional activity, while that from the light-uncaged protein was comparable to that from wild-type IL-2 (Figure 3d). Together, these data support that LED irradiation can derepress IL-2 latency, promote antigen-specific immunity, and reactivate JAK/STAT pathway signaling.

Feasibility of In Situ Cytokine Derepression. Having shown that cytokine latency can be used to temporally control immune cell signaling, we sought to characterize the spatial resolution with which cytokine activity could be constrained. We prepared a latent analog of IL-2 using bovine serum albumin modified with a photolabile PEG containing a biotin tag at its distal end (Figure 4a). Following immobilization onto glass slides, irradiation through a custom photolithographic mask, and streptavidin-FITC staining, we observed spatially constrained

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Figure 4. Light-induced uncaging enables precise, local control of protein activity. (a) Illustration of experiments to visualize protein latency and photoinduced derepression. (b) Fluorescence micrographs and (c) corresponding image line scan image indicating regions of latent (green) and uncaged (black) protein as measured by epifluorescence microscopy.

Figure 5. Latent IL-2 is stable and feasibly photoactivated through tissue models. (a) Illustration of multilayered tissue phantom construction. (b) Stability of 5 kDa poly(ethylene glycol) polymer photocages under prolonged, aqueous storage and under conditions simulating ambient, indoor light exposure of superficial veins (1 mm depth) as measured by cleavage-induced fluorescence dequenching. (c) Effect of tissue phantom light attention on latent IL-2 activity as measured by CTLL-2 T cell proliferation (24 h). (d) Superficial heating of multilayered tissue phantoms of the indicated thickness as measured by forward-looking infrared imaging. Data in panel (c) represent mean ± SD of three technical replicates. $p < .05$(*), $p < .01$(**), $p < .001$(***), $p < .0001$****).
protein uncaging with a resolution at or below the typical dimensions of single human immune cells (<<17 μm, Figure 4b,c). These results suggest that cytokine activity can be derepressed with high spatial and temporal control using this synthetic modification approach.

To explore the effects of tissue light attenuation on latent IL-2 activation, we fabricated a series of silicone-based phantoms that mimic light transmission through human dermis, epidermis, and hypodermis at wavelengths specific to the polymer photocages described here (Figure 5a). Using these models, we examined the stability of 5 kDa PEG photocages under prolonged, aqueous storage conditions and under conditions simulating ambient indoor light exposure of superficial veins (1 mm hypodermis depth). We observed high stability of aqueous solutions in cold storage with <10% total uncaging of polymer linkages after 90 days (Figure 5b). Given that many clinical products have postreconstitution shelf lives of just hours to days, the storage durations observed here appear sufficient for large-scale in vivo testing. We also observed comparably low levels of polymer cleavage under conditions mimicking nondeliberate light exposure of superficial veins over 6 weeks (<<4%). These latter data suggest that polymer-induced latency may be maintained in vivo over timescales necessary for light-constrained cytokine derepression.

To model the feasibility of light-induced cytokine uncaging in vivo, we examined the activity of latent IL-2 following photoexposure through tissue phantoms modeling human skin and subcutaneous tissue. We observed near full recovery of IL-2 activity at depths corresponding to 1 mm beneath the dermis as measured by CTLL-2 T cell proliferation (Figure 5c). These findings are significant as such depths, in many cases, correspond to the minimal light attenuation experienced at human superficial veins and within transcutaneous or some trans-epithelial tumors. Moreover, as the light irradiance required for activation through tissue phantoms induced only a small temperature increase (∆Tmax 10.0 °C, Figure 5d), heat pain responses in vivo are expected to be mild or imperceptible.

**DISCUSSION**

Inspired by the ability of latency-binding to impart specificity to otherwise pleiotropic immune signaling proteins, here, we describe a strategy whereby chemical modification with light-sensitive polymers can be used to control the activity of cytokines in response to simple LED light exposure. In this study, we found that modification of IL-2 and IL-15 with photolabile small molecules or polymers could modulate their activity on T cells as much as two to three orders of magnitude. This ability to control the magnitude, and correspondingly the duration, of IL-2 signals is significant as (i) both strong and sustained IL-2 signaling is necessary for the induction of the CD8⁺ effector—rather than memory—T cell fate, and (ii) as little as a tenfold change in local cytokine concentration can bias this tradeoff. While in vivo conditions, such as limited diffusion in tissue, extracellular matrix adhesion, and lymphatic fluid transport, may necessitate a wider range of light-induced activity modulation, strategies such as those described here may, in a future work, enable the optical reprogramming of fate decisions in T cells that lead to the short-term effector function at the expense of long-term memory function.

In this work, we also show that polymer-induced latency blunts corresponding JAK/STAT pathway signaling and CD8⁺ T cell activation ex vivo and that just brief LED light exposure can be used to derepress these effects. We envision that such high spatial and temporal control of cytokine signaling can be used to modulate T cell priming/expansion directly at sites of disease or at associated secondary lymphoid organs. Such strategies may also extend to chemokines, which can serve to further orchestrate effective adaptive immune responses against pathogens or tumors. Here, we achieved a minimum spatial resolution of photoactivation approaching that of a single immune cell—without the use of focusing optics—and although light scattering and diffusion would limit such dimensions in vivo, we anticipate strong feasibility to constrain cytokine activation to millimeter-scale diseased tissues and lymph nodes in future work. This supposition is also supported by tissue phantom studies performed here, showing efficient light-induced derepression at subdermal depths of as high as 1 mm, sufficient in many instances for activation within human superficial veins and within transcutaneous or some trans-epithelial disease sites. Others have also demonstrated that structurally related photocages appended to solid implants can be transcutaneously photoactivated in mice, thus we are optimistic regarding future in vivo feasibility; further testing, however, will be required to confirm recovery of activity in the present case and to also assess potential negative effects from prolonged near-UV light exposure, such as DNA damage, tissue fibrosis, or protein denaturation.

Serendipitously, we also found that polymer-induced latency, alone, biased the affinity of latent IL-2 toward IL-2Rαβγ (CD122) and away from IL-2Rαβγ (CD25). As CD8⁺ T cells express IL-2Rβγ and immunosuppressive Tregs constitutively express IL-2Rαβγ, these findings suggest that the latent cytokine may improve CD8⁺/Treg ratios, which are prognostically favorable in many cancers and correlate with clinical responses to an immune checkpoint blockade therapy in patients. While other mechanisms of cytokine receptor subunit-biasing based on mutagenesis, antibody complexation, and de novo protein design have been reported, here, we hypothesize that atypically high density of solvent-accessible lysine resides at the IL-2/IL-2Rαβγ interface preferentially induces steric hindrance with the receptor subunit via appended polymer chains.

In addition to demonstrating rapid and efficient cytokine photoactivation, we also found that polymer photocages used here were highly stable under conditions simulating both aqueous storage and venous ambient light exposure. Compared with other promising and more rapidly hydrolyzable PEG/IL-2 conjugates (e.g., NKTR-214), these findings are encouraging and could lead to future integration with wearable or implanted light-delivery devices, with other promising and more rapidly hydrolyzable PEG/IL-2 conjugates (e.g., NKTR-214), these findings are encouraging and could lead to future integration with wearable or implanted light-delivery devices, which modulate drug activation. Consistent with other PEGylated cytokines, we found that bioinspired, polymer-induced latency was able to prolong scIL-12 plasma circulation by approximately 11-fold, potentially precluding the need for frequent, high dosing and improving tissue drug exposure in treatment settings. Although the use of synthetic polymers such as PEG here provides many advantages including low cost and a track-record of use in more than 20 systemically administered clinical therapies or countless household products, it also presents some important limitations. The detection of anti-PEG antibodies among health individuals has increased markedly in recent years, from ~27% in 2003 to ~42% in 2015, and while pre-existing or drug-induced anti-PEG antibodies have been shown to correlate with infusion reactions and drug activity loss after multiple injections, such associations are often highly drug specific. Here, we employ recombinant human proteins, which are intrinsically weakly
immunogenic and a high degree of PEG modification in order to minimize such risks; however, future studies exploring alternative latency-inducing polymers or peptides may further improve drug activity modulation or production scalability.

Although these studies are the first, to our knowledge, to demonstrate reversible, optical control of cytokines, they build upon many prior advances. Deiters and co-workers previously demonstrated reversible photocaging strategies for enzymes based on nitrobenzyl linkages, while Esser-Kahn and co-workers and Lynn et al. have demonstrated related approaches to photoactivate smaller lipopeptide Toll-like receptor (TLR) agonists. Likewise, Garcia et al. Hubbell et al., and Wittrupp et al. have recently developed affinity-targeted cytokine fusions with a high cell- or tissue-specific activity. Combining elements of these approaches, here, we describe a bioinspired strategy for polymer-induced cytokine latency and photoinduced reactivation. The work presented here provides a proof of concept that cytokine activity can be precisely regulated using light, findings that may be further improved upon through the use of additional photolabile linkers, which absorb light at wavelengths with higher tissue penetration.

In summary, we describe a generalizable strategy for light-induced cytokine derepression that can be used to spatially and temporally control the activity of otherwise pleiotropic immune signaling molecules. As research tools, the technologies described here hold great potential to improve our ability to understand, manipulate basic immune biology, and optically reprogram immune responses ex vivo and in vitro. As therapies, they could also serve as long-acting prodrugs or tissue-selective immune modulators both alone or in combination with other immunotherapies.

**ASSOCIATED CONTENT**

1. **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.biomac.0c00264.

Characterization of linker cleavage, conjugation schematic, spectral properties of light sources and linker compounds, and latent IL-15 activity photomodulation (PDF)

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**Author Contributions**


**Notes**

The authors declare no competing financial interest.

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