adverse health outcomes, such as vascular leakage, through systemic immune activation. These findings illustrate the need for developing cytokine therapeutics that effectively target tumors without the side effects associated with systemic immune activation. Provided herein are masked cytokines, compositions thereof and methods of use thereof for addressing this need.

[0006] All references cited herein, including patent applications, patent publications, and scientific literature, are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0006a] In one aspect, provided herein is a masked cytokine comprising a first polypeptide H-linker-C and a second polypeptide H-linker-MM, wherein H is a Fc domain, C is an IL-12 cytokine, and MM comprises an extracellular domain of IL-12Rβ2; wherein a linker in the first polypeptide or a linker in the second polypeptide is cleavable; and wherein the Fc domain in the second polypeptide comprises Y349C, T366S, L368A and Y407 mutations and the Fc domain in the first polypeptide comprises S354C and T366W mutations relative to SEQ ID NO: 154 by EU numbering.

[0007] The disclosed invention relates to the engineering and use of masked cytokines or functional fragments thereof that, in some embodiments, are engineered to be masked by a masking moiety at one or more receptor binding site(s) of the cytokine or functional fragment thereof. In some embodiments, the cytokines are engineered to be activatable by a protease at a target site, such as in a tumor microenvironment, by including a proteolytically cleavable linker. In some embodiments, the proteolytically cleavable linker links the cytokine to the masking moiety, links the cytokine to a half-life extension domain, and/or links the masking moiety to a half-life extension domain. The masking moiety blocks, occludes, inhibits (e.g., decreases) or otherwise prevents (e.g., masks) the activity or binding of the cytokine to its cognate receptor or protein. Upon proteolytic cleavage of the cleavable linker at the target site, the cytokine becomes activated, which renders it capable of binding to its cognate receptor or protein with increased affinity.

[0008] One embodiment comprises a masked cytokine comprising a masking moiety; and a cytokine or functional fragment thereof, wherein the masking moiety is linked to the cytokine or functional fragment thereof via a linker. In one aspect, the masked cytokine further comprises a half-life extension domain that is linked to either the masking moiety or the cytokine or functional fragment thereof.

[0009] In one embodiment, the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a masking moiety; b) a first linker; c) a cytokine or functional fragment thereof; and d) a half-life extension domain. In another embodiment, the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a masking moiety; b) a first linker; c) a cytokine or functional fragment thereof; d) a second linker and e) a half-life extension domain. In another embodiment, the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a cytokine or functional fragment thereof; b) a first linker; c) a masking moiety; and d) a half-life extension domain.

[0010] In another embodiment, the masked cytokine comprises in an N to C-terminal or in a C to N-terminal direction: a) a cytokine or functional fragment thereof; b) a first linker; c) a masking moiety; d) a second linker; and e) a half-life extension domain.

rhinosinusitis, rhinosinusitis with polyps, eosinophilic esophogitis, eosinophilic bronchitis, Guillain-Barre disease, thyroiditis (e.g., Graves' disease), Addison's disease, Raynaud's phenomenon, autoimmune hepatitis, transplantation rejection, kidney damage, hepatitis C-induced vasculitis, or spontaneous loss of pregnancy.

[0037a] In another aspect, provided herein is use of a composition in the manufacture of a medicament for inhibiting tumor growth in a subject, wherein the composition comprises an effective amount of a masked cytokine comprising: a first polypeptide H-linker-C and a second polypeptide H-linker-MM, wherein H is a Fc domain, C vis an IL-12 cytokine, and MM comprises an extracellular domain of IL-12Rβ2; wherein a linker in the first polypeptide or a linker in the second polypeptide is cleavable; and wherein the Fc domain in the second polypeptide comprises Y349C, T366S, L368A, and Y407 mutations and the Fc domain in the first polypeptide comprises S354C and T366W mutations relative to SEQ ID NO: 154 by EU numbering, wherein the composition is to be administered to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] **FIG. 1A** and **FIG. 1B** show the structure of exemplary embodiments of a masked cytokine that includes a masking moiety, a cytokine or functional fragment thereof ("cytokine"), a half-life extension domain, and a first linker that includes a first cleavable peptide ("1CP"), a first N-terminal spacer domain ("1NSD"), and a first C-terminal spacer domain ("1CSD"). These exemplary embodiments also include a second linker that includes a second cleavable peptide ("2CP"), a second N-terminal spacer domain ("2NSD"), and a second C-terminal spacer domain ("2CSD"). As shown by the arrows, while the exemplary embodiments shows the masking moiety linked to the first linker, and the cytokine or functional fragment thereof is linked to the first linker and the second linker, the masking moiety and the cytokine or functional fragment thereof can be interchanged such that the cytokine or functional fragment thereof is linked to the first linker, and the masking moiety is linked to the first linker and the second linker. **FIG. 1A** shows the structure of an exemplary embodiment of a masked cytokine as a monomer. **FIG. 1B** shows the structure of an exemplary embodiment of a masked cytokine as a homodimer formed by disulfide bonds.

[0039] **FIG. 2A** and **FIG. 2B** show the structure of exemplary embodiments of a masked cytokine that includes a first masking moiety ("Mask 1"), a cytokine or functional fragment thereof ("cytokine"), a second masking moiety ("Mask 2"), a half-life extension domain, a first linker that includes a first cleavable peptide ("1CP"), a first N-terminal spacer domain ("1NSD"), and a first C-terminal spacer domain ("1CSD"), and a second linker that includes a second cleavable peptide ("2CP"), a second N-terminal spacer domain ("2NSD"), and a second C-terminal spacer domain ("2CSD"). These exemplary embodiments also include a third linker that includes a third cleavable peptide ("3CP"), a third N-terminal spacer domain ("3NSD"), and a third C-terminal spacer domain ("3CSD"). **FIG. 2A** shows the structure of an exemplary embodiment of a masked cytokine as a monomer. **FIG. 2B** shows the structure of an exemplary embodiment of a masked cytokine as a homodimer formed by disulfide bonds.

[0040] **FIG. 3A** shows the structure of an exemplary embodiment of a masked cytokine that includes a masking moiety, a cytokine or functional fragment thereof ("cytokine"), a first half-life extension domain, and a second half-life extension domain. The exemplary embodiment shown in **FIG. 3A** also includes a first linker that includes a first cleavable peptide ("1CP"), a first N-terminal spacer domain ("1NSD"), and a first C-terminal spacer domain ("1CSD"), and a second linker that includes a second cleavable peptide ("2CP"), a second N-terminal spacer domain ("2NSD"), and a second C-