[00134] Embodiment 124 is a host cell comprising the nucleic acid of embodiment 121 or the vector of embodiment 122.

[00135] Embodiment 125 is a method of producing a protease-activated pro-cytokine, comprising culturing the host cell of embodiment 124 under conditions wherein the protease-activated pro-cytokine is produced.

[00136] Embodiment 126 is the method of the immediately preceding embodiment, further comprising isolating the protease-activated pro-cytokine.

[00137] Embodiment 127 is a method of boosting T regulatory cells and/or reducing inflammation or autoimmune activity, comprising administering the protease-activated pro-cytokine of any one of embodiments 1-110 to an area of interest in a subject, e.g., an area of inflammation in the subject.

[00138] Embodiment 128 is a method of treating an inflammatory or autoimmune disease or disorder in a subject, comprising administering the protease-activated pro-cytokine of any one of embodiments 1-110 to an area of interest in a subject, e.g., an area of inflammation or autoimmune activity in the subject.

[00138a] In another embodiment, there is provided a protease-activated pro-cytokine comprising: a cytokine polypeptide sequence; an inhibitory polypeptide sequence capable of blocking an activity of the cytokine polypeptide sequence; a linker between the cytokine polypeptide sequence and the inhibitory polypeptide sequence, the linker comprising a protease-cleavable polypeptide sequence; and a targeting sequence, wherein the targeting sequence is configured to bind an extracellular matrix component, and the extracellular matrix component is heparin, collagen, or heparan sulfate proteoglycan.

[00138b] In another embodiment, there is provided a use of the protease-activated pro-cytokine as described herein in the manufacture of medicament for reducing inflammation or autoimmune activity.

[00138c] In another embodiment, there is provided a use of the protease-activated pro-cytokine as described herein in the manufacture of a medicament for treating an inflammatory or autoimmune disease or disorder in a subject.

FIGURE LEGENDS

[00139] **FIG 1A** shows an illustration of an exemplary cytokine prodrug structure and an SDS-PAGE gel characterizing a purified cytokine prodrug (Construct B). Abbreviations: PM, pharmacokinetic modulator; HMW, high molecular weight.

[00140] **FIG 1B** shows an illustration of an exemplary cytokine prodrug structure comprising human IL-2 and IL-2Rα sequences and an MMP-cleavable linker, and an SDS-PAGE gel and Western blot characterizing a purified cytokine prodrug (Construct E). Abbreviations: Hu, human; MMP, matrix metalloprotease; other abbreviations are as above.

[00141] **FIG 1C** shows an illustration of an exemplary cytokine prodrug structure comprising murine IL-2 and IL-2Rα sequences, an MMP-cleavable linker, additional linkers that include a targeting sequence ("RET Linker"), and an SDS-PAGE gel characterizing the indicated purified cytokine prodrugs.

[00142] **FIG 1D** shows an illustration of an exemplary cytokine prodrug structure comprising human IL-2 and IL-2Rα sequences, an MMP-cleavable linker, additional linkers that include a targeting sequence ("RET Linker"), and an SDS-PAGE gel characterizing the indicated purified cytokine prodrugs.

[00143] **FIG 2A** illustrates a cleavage reaction of a cytokine prodrug by a protease and shows Western blot evidence of cleavage of Construct A by MMP-9 at time points of 1, 2,

233	STWTWNGSAWTWNEGGK	Collagen		
234	STWTWNGTNWTRNDGGK	Collagen		WO/2014/059530
235	CVWLWEQC	Collagen		
236	CMTSPWRC	Collagen		Vanhoorelbeke, K., et al, J. Biol. Chem., 2003, 278, 37815-37821
237	CPGRVMHGLHLGDDEGPC	Collagen		Muzzard, J., et al, PLoS one. 4 (e5585) I- 10)
238	KLWLLPK	Collagen		Chan, J. M., et al, Proc Natl Acad Sci U.S.A., 2010, 107, 2213-2218)
239	CQDSETRTFY	Collagen		U.S. 2013/0243700
240	LSELRLHEN	Collagen		Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10984
241	LTELHLDNN	Collagen		Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10985
242	LSELRLHNN	Collagen		Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10986
243	LSELRLHAN	Collagen		Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10987
244	LRELHLNNN	Collagen		Fredrico, S., Angew. Chem. Int. Ed. 2015, 37, 10980-10988
245	RVMHGLHLGDDE	Collagen		
246	RVMHGLHLGNNQ	Collagen		
247	RVMHGLHLGNNQ	Collagen		
248	GQLYKSILYGSG-4K2K	Collagen	(a 4-branch peptide) which can be conjugated to a fusion polypeptide	
249	GSGQLYKSILY	Collagen		

We claim:

1. A protease-activated pro-cytokine comprising:

a cytokine polypeptide sequence;

an inhibitory polypeptide sequence capable of blocking an activity of the cytokine polypeptide sequence;

a linker between the cytokine polypeptide sequence and the inhibitory polypeptide sequence, the linker comprising a protease-cleavable polypeptide sequence; and

a targeting sequence, wherein the targeting sequence is configured to bind an extracellular matrix component, and the extracellular matrix component is heparin, collagen, or heparan sulfate proteoglycan.

2. The protease-activated pro-cytokine of claim 1, further comprising a pharmacokinetic modulator.

3. The protease-activated pro-cytokine of claim 2, wherein the pharmacokinetic modulator comprises an immunoglobulin constant domain; or

wherein the pharmacokinetic modulator comprises an immunoglobulin Fc region; preferably,

wherein the immunoglobulin is a human immunoglobulin; preferably,

wherein the immunoglobulin of the immunoglobulin Fc region or the human immunoglobulin is IgG; preferably,

wherein the IgG is IgG1, IgG2, IgG3, or IgG4.

4. The protease-activated pro-cytokine of claim 2, wherein the pharmacokinetic modulator comprises an albumin; preferably,

wherein the albumin is a serum albumin; preferably,

wherein the albumin or the serum albumin is a human albumin; or

wherein the pharmacokinetic modulator comprises PEG; or

wherein the pharmacokinetic modulator comprises XTEN; or

wherein the pharmacokinetic modulator comprises CTP.

5. The protease-activated pro-cytokine of any one of claims 2-4, wherein the protease-cleavable polypeptide sequence is between the cytokine polypeptide sequence and the pharmacokinetic modulator; or

wherein the pharmacokinetic modulator is between the cytokine polypeptide sequence and the protease-cleavable polypeptide sequence.

6. The protease-activated pro-cytokine of any one of claims 1-5, comprising a plurality of protease-cleavable polypeptide sequences; preferably,

wherein the cytokine polypeptide sequence is flanked by protease cleavable polypeptide sequences; preferably,

having the structure PM-CL-CY-CL-IN (from N- to C-terminus or from C- to N-terminus), where PM is the pharmacokinetic modulator, each CL independently is a protease-cleavable polypeptide sequence, CY is the cytokine polypeptide sequence, and IN is the inhibitory polypeptide sequence.

7. The protease-activated pro-cytokine of claim 6, comprising the targeting sequence, wherein the targeting sequence is between the cytokine polypeptide sequence and the protease-cleavable polypeptide sequence or one of the protease-cleavable polypeptide sequences; and/or

wherein the cytokine polypeptide sequence comprises a modification to prevent disulfide bond formation, and optionally otherwise comprises wild-type sequence; and/or

wherein the cytokine polypeptide sequence has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of a wild-type cytokine polypeptide sequence or to a cytokine polypeptide sequence selected from SEQ ID NOs: 1-4; and/or

wherein the cytokine polypeptide sequence is a wild-type cytokine polypeptide sequence; and/or

wherein the cytokine polypeptide sequence is a monomeric cytokine, or wherein the cytokine polypeptide sequence is a dimeric cytokine polypeptide sequence comprising monomers that are associated covalently, optionally via a polypeptide linker, or noncovalently; and/or

wherein the inhibitory polypeptide sequence comprises a cytokine-binding domain; preferably,

wherein the cytokine-binding domain is a cytokine-binding domain of a cytokine receptor or a cytokine-binding domain of a fibronectin; or

wherein the cytokine-binding domain is an immunoglobulin cytokine-binding domain; preferably,

wherein the immunoglobulin cytokine-binding domain comprises a light chain variable domain and a heavy chain variable domain that bind the cytokine; preferably,

wherein the immunoglobulin cytokine-binding domain or the immunoglobulin cytokine-binding domain comprising the light chain variable domain and the heavy chain variable domain that binds the cytokine is an scFv, Fab, or VHH.

8. The protease-activated pro-cytokine of any one of claims 1-7, wherein the protease-cleavable polypeptide sequence is recognized by a metalloprotease, a serine protease, a cysteine protease, an aspartate protease, a threonine protease, a glutamate protease, a gelatinase, an asparagine peptide lyase, a cathepsin, a kallikrein, a plasmin, a collagenase, a hKl, a hK10, a hK15, a stromelysin, a Factor Xa, a chymotrypsin-like protease, a trypsin-like protease, a elastase-like protease, a subtilisin-like protease, an actinidain, a bromelain, a calpain, a caspase, a Mir 1-CP, a papain, a HIV-1 protease, a HSV protease, a CMV protease, a chymosin, a renin, a pepsin, a matriptase, a legumain, a plasmepsin, a nepenthesin, a metalloexopeptidase, a metalloendopeptidase, an ADAM 10, an AD AMI 7, an ADAM 12, an urokinase plasminogen activator (uPA), an enterokinase, a prostate-specific target (PSA, hK3), an interleukin- lb converting enzyme, a thrombin, a FAP (FAP-a), a dipeptidyl peptidase, or dipeptidyl peptidase IV (DPPIV/CD26), a type II transmembrane serine protease (TTSP), a neutrophil elastase, a proteinase 3, a mast cell chymase, a mast cell tryptase, or a dipeptidyl peptidase; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of any one of SEQ ID NOs: 701-741, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 701-741; and/or

wherein the protease-cleavable polypeptide sequence is recognized by a matrix metalloprotease; and/or

wherein the protease-cleavable polypeptide sequence is recognized by MMP-1 or MMP-2 or MMP-3 or MMP-7 or MMP-8 or MMP-9 or MMP-12 or MMP-13 or MMP-14.

9. The protease-activated pro-cytokine of any one of claims 1-8, wherein the protease-cleavable polypeptide sequence is recognized by more than one MMP; and/or

wherein the protease-cleavable polypeptide sequence is recognized by two, three, four, five, six, or seven of MMP-2, MMP-7, MMP-8, MMP-9, MMP-12, MMP-13, and MMP-14.

10. The protease-activated pro-cytokine of any one of claims 1-9, wherein the protease-cleavable polypeptide sequence comprises the sequence of any one of SEQ ID NOs: 80-94 or

a variant sequence having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 80-90; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 80 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 81 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 82 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 83 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 84 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 85 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 86 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 87 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 88 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 89 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 90 or a variant sequence having one or two mismatches relative thereto; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 80-89 or 90; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 91; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 92; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 93; and/or

wherein the protease-cleavable polypeptide sequence comprises the sequence of SEQ ID NO: 94.

11. The protease-activated pro-cytokine of any one of claims 1-10, wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 180-276, 284-287, 592-628, 639, 640, 642-652, and 659-662, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 180-276, 284-287, 592-628, 639, 640, 642-652, and 659-662.

12. The protease-activated pro-cytokine of any one of claims 1-11, wherein the targeting sequence binds denatured collagen.

13. The protease-activated pro-cytokine of any one of claims 1-11, wherein the targeting sequence binds collagen.

14. The protease-activated pro-cytokine of claim 12 or 13, wherein the collagen is collagen I or collagen II or collagen III or collagen IV.

15. The protease-activated pro-cytokine of any one of claims 1-11, wherein the targeting sequence binds heparin.

16. The protease-activated pro-cytokine of any one of claims 1-11, wherein the targeting sequence binds heparan sulfate proteoglycan.

17. The protease-activated pro-cytokine of any one of claims 1-16, wherein the targeting sequence is configured to bind its target in a pH-sensitive manner; preferably,

wherein the targeting sequence has a higher affinity for its target at a pH below normal physiological pH than at normal physiological pH, optionally wherein the pH below normal physiological pH is below 7, or below 6; preferably,

wherein the targeting sequence has a higher affinity for its target at a pH in the range of 5-7, e.g., 5-5.5, 5.5-6, 6-6.5, or 6.5-7, than at normal physiological pH.

18. The protease-activated pro-cytokine of any one of claims 1-17, wherein the targeting sequence comprises one or more histidines, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 histidines; and/or

wherein the targeting sequence comprises the sequence of any one of SEQ ID NOs: 642-652 and 659-662, or a variant having one or two mismatches relative to the sequence of any one of SEQ ID NOs: 642-652 and 659-662.

19. The protease-activated pro-cytokine of any one of claims 1-10 and 15, wherein the targeting sequence comprises the sequence of VRIQRKKEKMKET.

20. The protease-activated pro-cytokine of any one of claims 1-19, wherein the cytokine polypeptide sequence is an interleukin polypeptide sequence; and/or

wherein the cytokine polypeptide sequence is capable of binding a receptor comprising CD132 or comprising CD122 or comprising CD25.

21. The protease-activated pro-cytokine of any one of claims 1-20, wherein the cytokine polypeptide sequence is an IL-2 polypeptide sequence; preferably,

wherein the IL-2 polypeptide sequence has at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of any one of SEQ ID NOs: 1-4; preferably,

wherein the IL-2 polypeptide sequence comprises the sequence of any one of SEQ ID NOs: 1-4.

22. The protease-activated pro-cytokine of claim 21, wherein the IL-2 polypeptide sequence is a human IL-2 polypeptide sequence; preferably,

wherein the IL-2 polypeptide sequence comprises the sequence of SEQ ID NO: 1; or

wherein the IL-2 polypeptide sequence comprises the sequence of SEQ ID NO: 2.

23. The protease-activated pro-cytokine of claim 21 or 22, wherein the inhibitory polypeptide sequence comprises an IL-2 binding domain of an IL-2 receptor (IL-2R); preferably,

wherein the inhibitory polypeptide sequence comprises an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of any one of SEQ ID NOs: 10-19; preferably,

wherein the IL-2R is a human IL-2R.

24. The protease-activated pro-cytokine of claim 21 or 22, wherein the inhibitory polypeptide sequence comprises an IL-2-binding immunoglobulin domain; preferably,

wherein the IL-2-binding immunoglobulin domain is a human IL-2-binding immunoglobulin domain; preferably,

wherein the IL-2-binding immunoglobulin domain comprises a VL region comprising hypervariable regions (HVRs) HVR-1, HVR-2, and HVR-3 having the sequences of SEQ ID NOs: 34, 35, and 36, respectively, and a VH region comprising HVR-1, HVR-2, and HVR-3 having the sequences of SEQ ID NOs: 37, 38, and 39, respectively.

25. The protease-activated pro-cytokine of claim 24, wherein the IL-2-binding immunoglobulin domain comprises a VL region comprising an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of SEQ ID NO: 32 and a

VH region comprising an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of SEQ ID NO: 33; preferably,

wherein the IL-2-binding immunoglobulin domain comprises a VL region comprising the sequence of SEQ ID NO: 32 and a VH region comprising the sequence of SEQ ID NO: 33.

26. The protease-activated pro-cytokine of claim 24, wherein the IL-2 -binding immunoglobulin domain is an scFv; preferably,

wherein the IL-2-binding immunoglobulin domain comprises an amino acid sequence having at least 80, 85, 90, 95, 97, 98, or 99 percent identity to the sequence of SEQ ID NO: 30 or 31; preferably,

wherein the IL-2-binding immunoglobulin domain comprises the sequence of SEQ ID NO: 30 or 31.

27. The protease-activated pro-cytokine of claim 1, comprising the sequence of any one of SEQ ID NOs: 809, 811-814, 817-821, 824, 825, 830-836, and 848-851.

28. A pharmaceutical composition comprising the protease-activated pro-cytokine of any one of claims 1-27.

29. The protease-activated pro-cytokine of any of claims 1-27 or the pharmaceutical composition of claim 28, for use in therapy.

30. Use of the protease-activated pro-cytokine of any of claims 1-27 or the pharmaceutical composition of claim 28 in the manufacture of a medicament for treating a cancer.

31. The use of claim 30, wherein:

the cancer is a solid tumor;

the cancer is a metastatic and/or unresectable solid tumor;

the cancer is a PD-L1-expressing cancer;

the cancer is a melanoma, a colorectal cancer, a breast cancer, a pancreatic cancer, a lung cancer, a prostate cancer, an ovarian cancer, a cervical cancer, a gastric or gastrointestinal cancer, a lymphoma, a colon or colorectal cancer, an endometrial cancer, a thyroid cancer, or a bladder cancer;

the cancer is a microsatellite instability-high cancer; and/or

the cancer is mismatch repair deficient.

32. A nucleic acid encoding the protease-activated pro-cytokine of any one of claims 1-27.

33. An expression vector comprising the nucleic acid of claim 32.

34. A host cell comprising the nucleic acid of claim 32 or the expression vector of claim 33.

35. A method of producing a protease-activated pro-cytokine, comprising culturing the host cell of claim 34 under conditions wherein the protease-activated pro-cytokine is produced; preferably,

further comprising isolating the protease-activated pro-cytokine.

36. Use of the protease-activated pro-cytokine of any one of claims 1-27 in the manufacture of medicament for reducing inflammation or autoimmune activity.

37. Use of the protease-activated pro-cytokine of any one of claims 1-27 in the manufacture of a medicament for treating an inflammatory or autoimmune disease or disorder in a subject.