HtrA1 promoter region, which results in increased HtrA1 transcript levels (Dewan, A., et al., *Science* 314:989-92 (2006); Yang, Z., et al., *Science* 314:992-3 (2006)). Therefore, inhibition of HtrA1 enzymatic function is an attractive therapeutic approach, e.g. in age-related macular degeneration and in arthritic disease.

SUMMARY

The disclosure provides anti-HtrA1 antibodies and methods of using the same for diagnostic and therapeutic purposes.

In one aspect, the invention provides an isolated monoclonal antibody that binds to HtrA1 competitively with an antibody comprising a VH sequence of SEQ ID NO:8 and a VL sequence of SEQ ID NO:7. Competitive binding may be determined, for example, using an ELISA assay.

In one embodiment, the disclosure provides an isolated antibody that bind to HtrA1 having one or more of the following properties: (i) an IC_{50} of less than 50 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 5 nM, 3 nM, 2.5 nM, 2 nM, 1 nM, or less for one or more HtrA1 substrates; (ii) binds to HtrA1 with a ratio of 1 variable domain to one subunit of an HtrA1 trimer (e.g., a Fab binds to an HtrA1 trimer with a ratio of 3 Fab to 1 HtrA1 trimer, and an IgG binds to an HtrA1 trimer with a ratio of 3 IgG to 2 HtrA1 trimers), (iii) for antibodies comprising two variable domains, binds to HtrA1 in a manner that results in the forming a "cage" similar to that shown in Fig. 9, (iv) does not prevent trimer formation of HtrA1, (v) binds to one or more residues in Loop C of the HtrA1 protein, (vi) binds to the protease domain of HtrA1, (vii) binds to an epitope comprising one or both of amino acids N224 or K248 of SEQ ID NO:13, or amino acids equivalent thereto in a different HtrA1 sequence (e.g., amino acids N224 and K248 of SEQ ID NO:14, see Figs. 10A and B); (viii) binds to an epitope comprising one or more of the following residues of N224, K248, V201, T223, K243, K225, E247 and H220 of SEQ ID NO:13, or amino acids equivalent thereto in a different HtrA1 sequence; (ix) cross-reacts with murine HtrA1; (x) does not cross-react with HtrA2, HtrA3 and/or HtrA4; (xi) binds to HtrA1 competitively with an antibody comprising a VH sequence of SEQ ID NO:8 and a VL sequence of SEQ ID NO:7, (xii) binds to HtrA1 with a dissociation constant of $\leq$500 nM, or (xiii) inhibits complex formation between HtrA1 and α1-antitrypsin (A1AT).

Also provided is an isolated antibody that binds to HtrA1, wherein the antibody (i) binds to an epitope that includes N224, K248, or both of HtrA1, and (ii) inhibits HtrA1 with an IC_{50} of $\leq$ 30 nM. In one aspect, the invention provides an isolated monoclonal antibody comprising a heavy chain variable domain (VH) and a light chain variable domain (VL) that bind to HtrA1, wherein the antibody (i) binds to an epitope that includes N224, K248, or both of HtrA1, and (ii) inhibits HtrA1 with a dissociation constant of $\leq$500 nM. In certain embodiments, the epitope further includes one or more of the following residues of HtrA1: V201, T223, K243, K225, E247 and H220. In certain embodiments, the antibody may further comprise one or more of the

WHAT IS CLAIMED IS:

1. An isolated monoclonal antibody comprising a heavy chain variable domain (VH) and a light chain variable domain (VL) that bind to HtrA1, wherein the antibody (i) binds to an epitope that includes N224, K248, or both of HtrA1, and (ii) inhibits HtrA1 with a dissociation constant of ≤500 nM.

2. The antibody of claim 1, wherein the antibody further comprises one or more of the following properties: (i) binds to HtrA1 with a ratio of one variable domain to one subunit of an HtrA1 trimer, or (ii) does not prevent trimer formation of HtrA1.

3. The antibody of claim 1 or 2, wherein the antibody does not cross-react with one or more of HtrA2, HtrA3 and HtrA4.

4. The antibody of claim 1, wherein the dissociation constant is determined by BIAcore using a Fab at 25 °C.

5. An isolated monoclonal antibody that binds to HtrA1 competitively with an antibody comprising a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7.

6. The antibody of claim 5, wherein competitive binding is determined using an ELISA assay.

7. The antibody of any one of claims 1-6, which is:
   (i) an antibody which is a human, humanized, or chimeric antibody, or
   (ii) an antibody which is an antibody fragment comprising a VH and a VL that bind HtrA1.

8. The antibody of any one of claims 1-7, wherein the antibody comprises (a) HVR-H3 comprising the amino acid sequence GTFLTX$_p$WGHYFDY, wherein X$_p$ is S or T (SEQ ID NO: 27); (b) HVR-L3 comprising the amino acid sequence QQX$_g$X$_h$X$_i$X$_j$PX$_k$T, wherein X$_g$ is S, V or D; X$_h$ is Y, D or S; X$_i$ is T, S, A, D or N; X$_j$ is T, H, N, S, A, L or R; and X$_k$ is P, T, A or S (SEQ ID NO: 24); and (c) HVR-H2 comprising the amino acid sequence WIDPYGGDTX$_0$YADSVKG, wherein X$_0$ is N or D (SEQ ID NO: 26).

9. The antibody of any one of claims 1-8, selected from a group consisting of:
   (i) an antibody which comprises (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6; (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 3; and
(c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5,

(ii) an antibody which comprises (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6; (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19; and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5,

(iii) an antibody which comprises (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6; (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 22; and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5, and

(iv) an antibody which comprises (a) HVR-H1 comprising the amino acid sequence $GFX_lIX_mX_nYYIH$, wherein X_l is N, S or T; X_m is S, D, Y or A; and X_n is G or D (SEQ ID NO: 25); (b) HVR-H2 comprising the amino acid sequence $WIDPYGGDTX_0YADSVKG$, wherein X_0 is N or D (SEQ ID NO: 26); and (c) HVR-H3 comprising the amino acid sequence $GTFLTX_pWGHYFDY$, wherein X_p is S or T (SEQ ID NO: 27).

10. The antibody of any one of claims 1-8 or 9(iv), further comprising (a) HVR-L1 comprising the amino acid sequence $RASQX_aX_bX_cX_dX_eX_fA$, wherein X_a is D, S or V; X_b is V or I; X_c is S, N or G; X_d is T or N; X_e is A or Y; and X_f is V or L (SEQ ID NO: 23); (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (c) HVR-L3 comprising the amino acid sequence $QQX_gX_hX_iX_jPX_kT$, wherein X_g is S, V or D; X_h is Y, D or S; X_i is T, S, A, D or N; X_j is T, H, N, S, A, L or R; and X_k is P, T, A or S (SEQ ID NO: 24).

11. The antibody of any one of claims 1-8, 9(iv) or 10, selected from a group consisting of:

(i) an antibody which comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 4; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6,

(ii) an antibody which comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6,

(iii) an antibody which comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 1; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 3,

(iv) an antibody which comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 18; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19, and

(v) an antibody which comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 22.

12. The antibody of claim 11(i), further comprising (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 1; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 3.

13. The antibody of claim 11(ii), selected from a group consisting of:

an antibody that further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 18; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19; and

an antibody that further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 22.

14. The antibody of any one of claims 1-13, further comprising a heavy chain variable domain framework (FR2) sequence of SEQ ID NO: 17.

15. The antibody of any one of claims 1-14, wherein (a) the VH sequence has at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 8; (b) the VL sequence has at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 7; or (c) the VH sequence has at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 8 and the VL sequence has at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 7.

16. The antibody of any one of claims 1-15, wherein (a) the VH sequence comprises the amino acid sequence of SEQ ID NO: 32; (b) the VL sequence comprises the amino acid sequence of SEQ ID NO: 31; or (c) the VH sequence comprises the amino acid sequence of SEQ ID NO: 32 and the VL sequence comprises the amino acid sequence of SEQ ID NO: 31.

17. The antibody of claim 15 or 16, wherein:

(i) the VH sequence is the amino acid sequence of SEQ ID NO: 8,

(ii) the VL sequence is the amino acid sequence of SEQ ID NO: 7,

(iii) the VL sequence is the amino acid sequence of SEQ ID NO: 30,

(iv) the VL sequence is the amino acid sequence of SEQ ID NO: 28; or

(v) the VH sequence is the amino acid sequence of SEQ ID NO: 29.

18. An antibody comprising a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 7.

19. An antibody comprising a VH sequence of SEQ ID NO: 29 and a VL sequence of SEQ ID NO: 28.

20. An antibody comprising a VL sequence of SEQ ID NO: 30 and a VH sequence of SEQ ID NO: 29.

21. The antibody of any one of claims 1-7, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence $GFX_lIX_mX_nYYIH$, wherein X_l is N, S or T; X_m is S, D, Y or A; and X_n is G or D (SEQ ID NO: 25); (b) HVR-H2 comprising the amino acid sequence $WIDPYGGDTX_0YADSVKG$, wherein X_0 is N or D (SEQ ID NO: 26); (c) HVR-H3 comprising the amino acid sequence $GTFLTX_pWGHYFDY$, wherein X_p is S or T (SEQ ID NO: 27); (d) HVR-L1 comprising the amino acid sequence $RASQX_aX_bX_cX_dX_eX_fA$, wherein X_a is D, S or V; X_b is V or I; X_c is S, N or G; X_d is T or N; X_e is A or Y; and X_f is V or L (SEQ ID NO: 23); (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (f) HVR-L3 comprising the amino acid sequence $QQX_gX_hX_iX_jPX_kT$, wherein X_g is S, V or D; X_h is Y, D or S; X_i is T, S, A, D or N; X_j is T, H, N, S, A, L or R; and X_k is P, T, A or S (SEQ ID NO: 24).

22. The antibody of claim 21, wherein the antibody comprises (a) HVR-H1 comprising an amino acid sequence selected from SEQ ID NOs: 4, 20, and 47-51; (b) HVR-H2 comprising an amino acid sequence selected from SEQ ID NOs: 5 and 52; (c) HVR-H3 comprising an amino acid sequence selected from SEQ ID NOs: 6 and 53; (d) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 1, 18, 21, and 33; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NOs: 3, 19, 22, and 34-46.

23. The antibody of claim 22, selected from a group consisting of:

(i) an antibody which comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 4; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 1; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 3,

(ii) an antibody which comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 18; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19, and

(iii) an antibody which comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 5; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 2; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 22.

24. The antibody of any one of claims 1-23, which is a full-length IgG1 antibody.

25. The antibody of any one of claims 1-23, which is a full-length IgG4 antibody.

26. An isolated nucleic acid encoding the antibody of any one of claims 1-25.

27. A host cell comprising the nucleic acid of claim 26.

28. A method of producing an antibody comprising culturing the host cell of claim 27 under conditions suitable for expression of the nucleic acid encoding the anti-HtrA1 antibody.

29. The method of claim 28, further comprising recovering the anti-HtrA1 antibody from the host cell culture.

30. An immunoconjugate comprising the antibody of any one of claims 1-25 and a cytotoxic agent.

31. A pharmaceutical formulation comprising the antibody of any one of claims 1-25 and a pharmaceutically acceptable carrier.

32. The antibody of any one of claims 1-25 for use as a medicament.

33. Use of the antibody of any one of claims 1-25 in the manufacture of a medicament.

34. The use of claim 33, selected from a group consisting of:

(i) a use wherein the medicament is for treatment of treating age-related macular degeneration, geographic atrophy, diabetic retinopathy, retinopathy of prematurity, or polypoidal choroidal vasculopathy,

(ii) a use wherein the medicament is for inhibiting degeneration of retinal or photoreceptor cells, and

(iii) a use wherein the medicament is for inhibiting HtrA1 protease activity in an eye.

35. The use of claim 34(i), wherein the age-related macular degeneration is dry age-related macular degeneration.

36. The antibody of any one of claims 1-25, wherein the antibody binds to HtrA1 and inhibits its serine protease activity for one or more HtrA1 substrates.