

Table 1: Immune Cells in Human Blood*

Cell type	Median Concentration in human blood [Cells x 10 ⁹ /L]	Median percentage of white blood cells
Neutrophils	3.65	53.0
Lymphocytes (incl. T cells, B cells and NK cells)	2.5	36.1
Eosinophils	0.15	3.2
Basophils	0.03	0.6
Monocytes	0.43	7.1

*Data collected from 291 adults (see Orfanakis, *et al.*, 1970, *Am J Clin Pathol*, 53:647-651)

Recruitment and activation of neutrophils via IgA affords new biological functions for antibody-based immunotherapies.

5 SUMMARY

In one aspect, provided herein is an IgA heterodimeric Fc (IgA HetFc) construct comprising a first Fc polypeptide and a second Fc polypeptide, the first Fc polypeptide comprising a first CH3 domain sequence and the second Fc polypeptide comprising a second CH3 domain sequence, the first and second CH3 domain sequences forming a modified CH3 domain,

10 wherein the first and second CH3 domain sequences comprise amino acid mutations that promote formation of a heterodimeric Fc over a homodimeric Fc,

wherein:

15 the amino acid mutations in the first CH3 domain sequence comprise an amino acid substitution at position A6085Y selected from A6085YF, A6085YY, A6085YM, A6085YW and A6085YH, and an amino acid substitution at position T6086 selected from T6086Y, T6086F, T6086M, T6086W and T6086H, and

the amino acid mutations in the second CH3 domain sequence comprise an amino acid substitution at position W6081 selected from W6081T, W6081L, W6081A, W6081V and W6081I,

wherein the heterodimeric Fc is formed with a purity of 70% or higher,

and wherein the numbering of amino acid positions is according to IMGT numbering.

In another aspect, provided herein is an IgA heterodimeric Fc (IgA HetFc) construct comprising a first Fc polypeptide and a second Fc polypeptide, the first Fc polypeptide comprising a first CH3 domain sequence and the second Fc polypeptide comprising a second CH3 domain sequence, the first and second CH3 domain sequences forming a modified CH3 domain,

wherein the first and second CH3 domain sequences comprise amino acid mutations that promote formation of a heterodimeric Fc over a homodimeric Fc,

wherein:

(a) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YY and T6086L, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079T, W6081L and I6088L; or

(b) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YY and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079T, W6081L and I6088L; or

(c) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081L and I6088L; or

(d) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(e) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: T6022V, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(f) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: T6022L, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(g) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: T6022I, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

5 (h) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6007F, L6079V, W6081T and I6088L; or

(i) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: H6005Y, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: H6005Y, L6079V, W6081T and I6088L;
10 or

(j) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: H6005C, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: P6010C, L6079V, W6081T and I6088L;
or

15 (k) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: P6010C, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: H6005C, L6079V, W6081T and I6088L;
or

(l) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: H6005C, P6010C, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: H6005C, P6010C, L6079V, W6081T and I6088L,
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and wherein the numbering of amino acid positions is according to IMGT numbering.

In another aspect, provided herein is an IgA heterodimeric Fc (IgA HetFc) construct
25 comprising a first Fc polypeptide and a second Fc polypeptide, the first Fc polypeptide comprising a first CH3 domain sequence and the second Fc polypeptide comprising a second CH3 domain sequence, the first and second CH3 domain sequences forming a modified CH3 domain,

wherein the first and second CH3 domain sequences comprise amino acid mutations that promote formation of a heterodimeric Fc over a homodimeric Fc,

wherein:

(a) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

5 (b) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6007F, L6079V, W6081T and I6088L; or

(c) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: H6005Y, A6085YF and T6086Y, and the amino acid mutations in the second CH3
10 domain sequence comprise the amino acid substitutions: H6005Y, L6079V, W6081T and I6088L,

wherein the heterodimeric Fc is formed with a purity of 70% or higher,

wherein the modified CH3 domain has a melting temperature (T_m) that is 60°C or higher,

and wherein the numbering of amino acid positions is according to IMGT numbering.

Described herein are heterodimeric IgA Fc constructs and methods of use thereof. One
15 example of the present disclosure relates to an IgA heterodimeric Fc (IgA HetFc) construct comprising a first Fc polypeptide and a second Fc polypeptide, the first Fc polypeptide comprising a first CH3 domain sequence and the second Fc polypeptide comprising an second CH3 domain sequence, the first and second CH3 domain sequences forming a modified CH3 domain, wherein the first and second CH3 domain sequences comprise amino acid mutations that promote formation
20 of a heterodimeric Fc over a homodimeric Fc, wherein: the amino acid mutations in the first CH3 domain sequence comprise an amino acid substitution at position A6085Y selected from A6085YF, A6085YY, A6085YM, A6085YW and A6085YH, and an amino acid substitution at position T6086 selected from T6086Y, T6086F, T6086M, T6086W and T6086H, and the amino acid mutations in the second CH3 domain sequence comprise an amino acid substitution at position
25 W6081 selected from W6081T, W6081L, W6081A, W6081V and W6081I, wherein the heterodimeric Fc is formed with a purity of 70% or higher, and wherein the numbering of amino acid positions is according to IMGT numbering.

Another example of the present disclosure relates to an IgA heterodimeric Fc (IgA HetFc) construct comprising a first Fc polypeptide and a second Fc polypeptide, the first Fc polypeptide comprising a first CH3 domain sequence and the second Fc polypeptide comprising an second CH3 domain sequence, the first and second CH3 domain sequences forming a modified CH3 domain, wherein the first and second CH3 domain sequences comprise amino acid mutations that promote formation of a heterodimeric Fc over a homodimeric Fc, wherein:

(a) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YY and T6086L, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079T, W6081L and I6088L; or

(b) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YY and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079T, W6081L and I6088L; or

(c) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081L and I6088L; or

(d) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(e) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: T6022V, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(f) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: T6022L, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(g) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: T6022I, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or