

acids 94-107 of SEQ ID NO: 1), the loop2 region (amino acids 172-202 of SEQ ID NO: 1) and the loop3 region (amino acids 264-280 of SEQ ID NO: 1) are substituted with the corresponding regions of human CCR4, the N-terminal region (amino acids 1-39 of SEQ ID NO: 48), the loop1 region (amino acids 98-111 of SEQ ID NO: 48), the loop2 region (amino acids 176-206 of SEQ ID NO: 48) and the loop3 region (amino acids 268-284 of SEQ ID NO: 48), respectively, were produced, and binding of each antibody was evaluated by a method similar to that described above.

Binding at 5 ug/mL, 0.5 ug/mL, and 0.05 ug/mL was evaluated, and, in comparison to wild-type human CCR8 (hCCR8), an antibody having significantly reduced binding activity was indicated as Δ and an antibody having binding activity of the detection limit or less was indicated as $\times$. The blank column means that it exhibits the same binding activity as the wild-type human CCR8 (hCCR8).

As a result, as shown in Table 2, all antibodies having neutralizing activity had the binding activity of the detection limit or less by substituting the N-terminal region with human CCR4 (N-ter hCCR4-hCCR8), thus it was revealed that the N-terminal region is an important epitope for exerting neutralizing activity. In addition, the binding activities to loop1-human CCR4-substituted human CCR8 and loop2-human CCR4-substituted human CCR8 were also reduced. From the above, it is considered that anti-CCR8 antibodies having strong neutralizing activity have stereo-structural recognition to strongly recognize the N-terminal region and bind to loop1 and loop2.

Note that although an antibody that does not recognize any of human CCR8, human CCR4, and mouse CCR8 was used as a control, the control antibody did not bind to each antigen. It was also confirmed that expression of hCCR8 has no reduction due to mutation by attaching a tag to the N-terminal of each antigen and detecting it with an anti-tag antibody.

Furthermore, to analyze in more detail the N-terminal region that is an epitope region common to all clones having strong neutralization, the human CCR8 point mutants shown in Table 2 were produced. As a result of evaluating the binding activity of each clone to the mutants by a similar method to the method described above, all 27 neutralizing antibodies described above had significantly reduction to the detection limit or less in their binding activities to the mutant in which Y at position 17 of human CCR8 is substituted for A (hCCR8 (Y17A)). It is thus considered that the neutralizing antibodies recognize Y at position 17. In addition, although clone No. 8F7 is only shown in Table 2, plural other neutralizing antibodies had reduced binding activity to the mutant in which I at position 20 of human CCR8 is substituted with A (hCCR8 (I20A)). It is thus considered that some neutralizing antibodies have stereo-structural recognition to bind to isoleucine at position 20.

Meanwhile, 13 non-neutralizing antibodies described above did not reduce their binding activities to hCCR8 (Y17A), thus it is considered that they recognize the same N-terminal region, but not Y at position 17. From the above, it is considered that Y at position 17 is an amino acid that is extremely important for exerting neutralizing activity. The results for representative neutralizing antibodies (clone Nos. 10A11, 27G1, 1H4, 8F7, 2C7) and non-neutralizing antibodies (clone No. 5B5) are shown in Table 2.

[0086]

[Table 2]

clone No.	10A11	27G1	1H4	8F7	2C7	5B5*
Neutralizing Activity IC50 (nM)	0.08	0.09	0.32	1.56	0.40	>1000
hCCR8						
hCCR4	x	x	x	x	x	x
mCCR8	x	x	x	x	x	x
N-ter hCCR4-hCCR8	x	x	x	x	x	Δ
loop1 hCCR4-hCCR8	Δ	Δ	Δ	x	x	
loop2 hCCR4-hCCR8	Δ	Δ	Δ	Δ	Δ	
loop3 hCCR4-hCCR8						
hCCR8 (L5A)			Δ		Δ	
hCCR8 (D6A)						
hCCR8 (L7A)						
hCCR8 (S8A)						
hCCR8 (T10A)					Δ	
hCCR8 (T11A)			Δ			
hCCR8 (V12A)						
hCCR8 (T13A)						
hCCR8 (Y15A)						
hCCR8 (Y16A)						
hCCR8 (Y17A)	x	x	x	x	x	
hCCR8 (I20A)				Δ		
hCCR8 (S22A)					Δ	
hCCR8 (S23A)						
hCCR8 (L29A)						
hCCR8 (I30A)				Δ		
hCCR8 (Q31A)						
hCCR8 (T32A)						
hCCR8 (N33A)						
hCCR8 (G34A)						
hCCR8 (K35A)			x	Δ		

*: non-neutralizing antibodies

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Example 4: Determination of antibody sequences