METHODS

Human subjects

AA and NA subjects were classified based on physical examination, history, increased blood IgE levels (>50 IU/mL for allergic asthma), and positive skin tests to allergens (for AA subjects). HC subjects were defined as nonsmoking subjects greater than 13 years of age with a total serum IgE level of less than 25 IU/mL, negative skin test results (compared with positive histamine control), and no evidence of lung disease. In addition, there was no evidence of obstructive or restrictive lung disease for HC subjects on spirometric testing. Patients with FEV_1 values of less than 60% were considered to have severe disease. Those in the range of 60% to 80% were considered to have moderate disease, and those with FEV_1 values of greater than 80% were considered to have mild disease. Disease duration and disease severity match between NA and AA subjects was attempted. Age match for all clinical groups was also attempted as much as possible.

Cell isolation

The $CD4^+$ T-cell fraction was first incubated with CD25 microbeads (clone BC96; Miltenyi Biotec, Bergisch Gladbach, Germany) to positively isolate $CD4^+CD25^+$ T cells. This fraction was subsequently stained with CD25–phycoerythrin (clone 4E3, Miltenyi Biotec) and CD127–allophycocyanin (clone SB199; BioLegend, San Diego, Calif) antibodies and eventually sorted for $CD4^+CD25^{hi}CD127^{lo/-}$ Treg cells. The flowthrough fraction after magnetic purification contained $CD4^+CD25^-$ cells (non-Treg cells). Cells were rested in RPMI, 10% FBS, and 1% L-glutamine after purification for 2 hours before undergoing downstream assays. The purity of each fraction was confirmed to be more than 95% with Foxp3 staining (clone 206D, BioLegend). All procedures were performed with manufacturers' standard protocols.

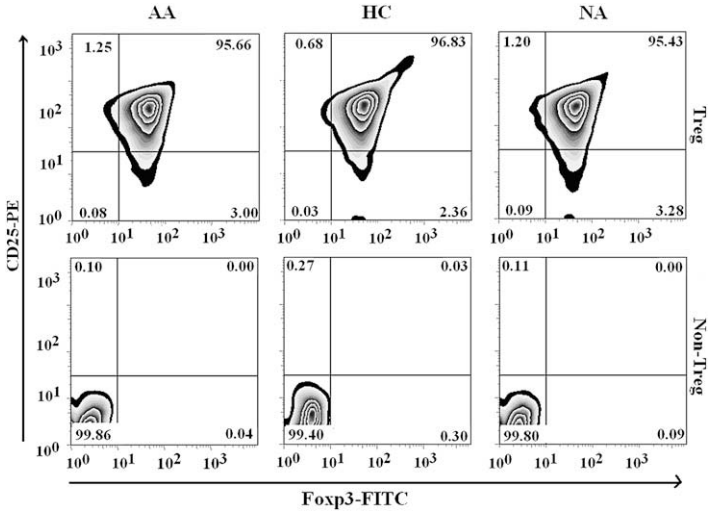

FIG E1. Purity of Treg and non-Treg cells. Expression of CD25 and Foxp3 by purified Treg and non-Treg cells in representative AA, HC, and NA subjects. *PE*, Phycoerythrin; *FITC*, fluorescein isothiocyanate.

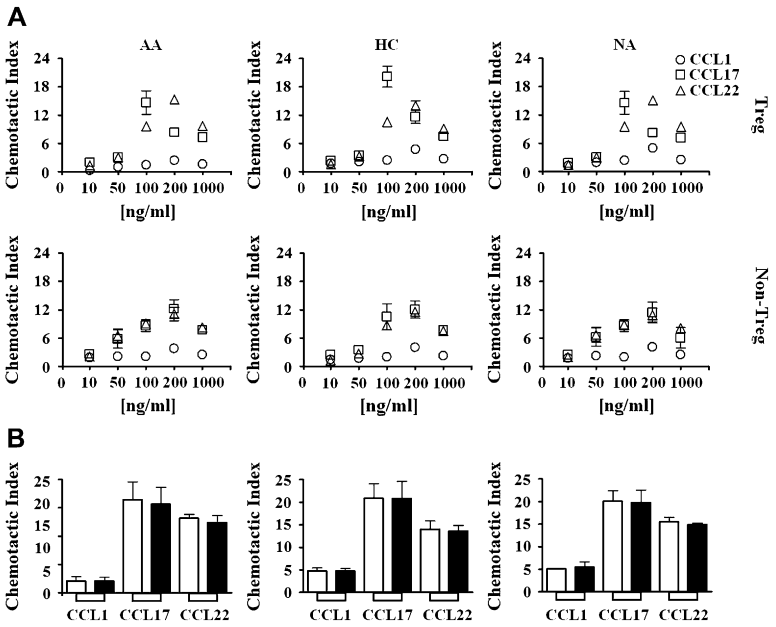

FIG E2. Titration of chemokines used in chemotaxis assays. **A**, Titration of chemokine dosage for each clinical group. **B**, Comparison between chemotactic responses of flow cytometry–sorted Treg cells *(open bars)* and flow cytometry– and magnetic bead–sorted Treg cells *(solid bars)* to CCL1, CCL17, and CCLL22 in each clinical group. Data represented means and SDs from 3 and 5 representatives from each clinical group in Fig E2, *A* and *B*, respectively. In Fig E2, *B*, the Wilcoxon test was used for paired analyses because the data were not normally distributed.

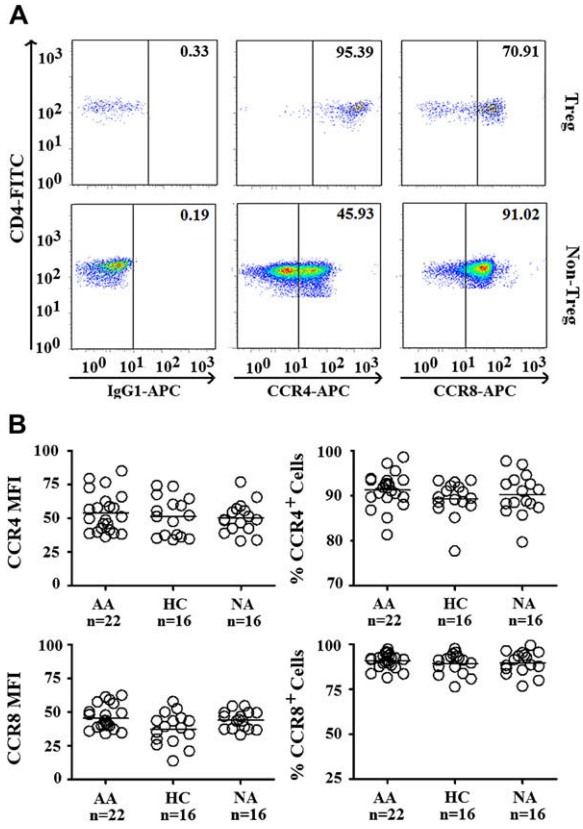

FIG E3. CCR4 and CCR8 expression in Treg and non-Treg cells. **A**, Gating of $CCR4^+$ cells and $CCR8^+$ cells in Treg and non-Treg cells. Negative thresholds for CCR4 and CCR8 expression by Treg and non-Treg cells were derived from isotype controls. *FITC*, Fluorescein isothiocyanate; *APC*, allophycocyanin. **B**, CCR4 and CCR8 expression by non-Treg cells. The Kruskal-Wallis test was used for the analysis of the percentages of $CCR4^+$ cells because the data were not normally distributed. ANOVA was used for the rest of the analyses because the data had normal distributions and equal variances. *MFI*, Mean fluorescence intensity.

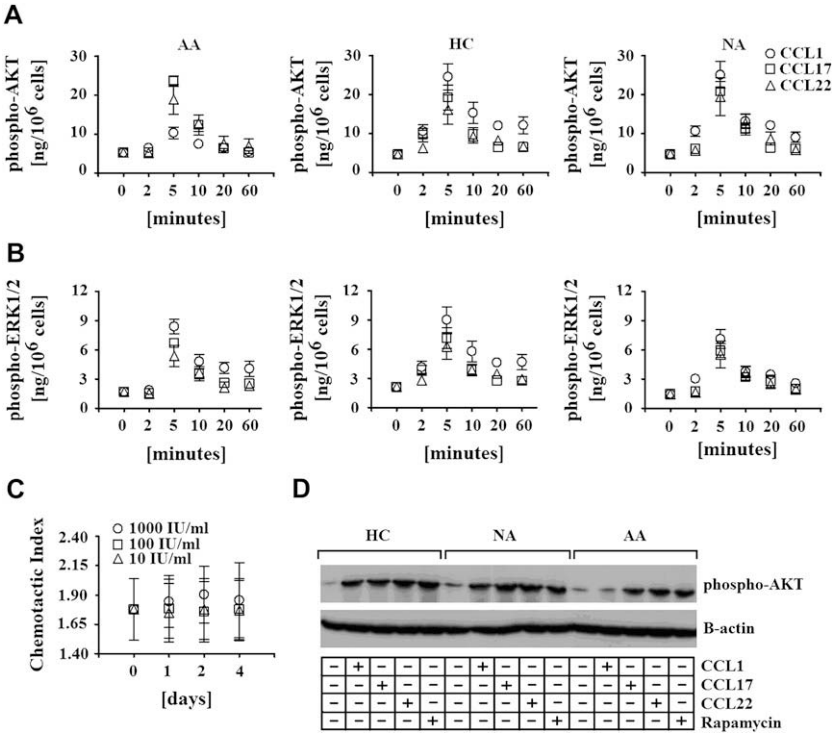

FIG E4. Phosphoprotein analysis of chemokine signaling pathways. **A**, Kinetics of AKT phosphorylation in response to CCL1, CCL17, and CCL22 in each clinical group. **B**, Kinetics of ERK1/2 phosphorylation in response to CCL1, CCL17, and CCL22 in each clinical group. **C**, Titration of IL-2 dosage and kinetics of IL-2 incubation with respect to its effects on CCL1 dysfunction in AA Treg cells. Data represented means and SDs from 3 representatives from each clinical group. "0" time points mean baseline chemotactic responses without any IL-2 supplement or any added chemokines. **D**, Expression of phospho-AKT in Treg cells from AA, NA, and HC representatives in response to CCL1, CCL17, CCL22, and rapamycin (positive control). Unstimulated samples were used as negative controls. β-Actin expression was used to control for equal loading. Optimal concentrations and stimulation times used in Western blot experiments were derived from previous phospho-ELISA works.

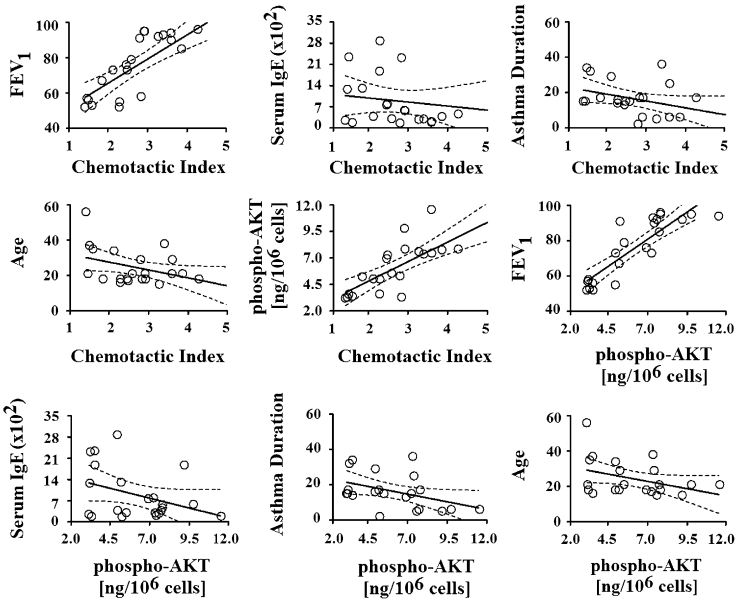

FIG E5. Correlation of *in vitro* cell functions and clinical parameters of allergic asthma. The Spearman test was used for correlation analyses of nonnormally distributed data. *Dotted lines* represent the 95% CIs. *Solid lines* represented the best-fitted lines of the data.

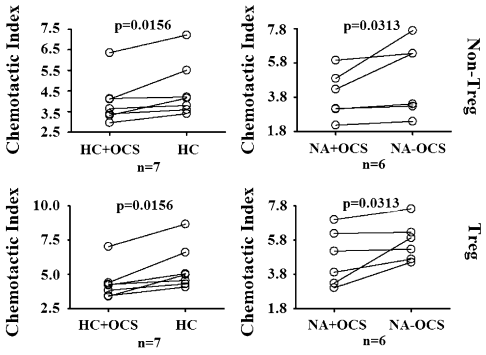

FIG E6. Effects of oral corticosteroids *(OCS)* on CCL1 chemotactic responses of Treg and non-Treg cells from HC and NA subjects. Comparisons were made between cells from the same NA subjects that were collected with and without steroid withdrawal enforcement. HC cells were incubated with 1000 ng/mL OCS for 24 hours before being subjected to downstream assays. The Wilcoxon test was used for the paired analysis of nonnormally distributed data.

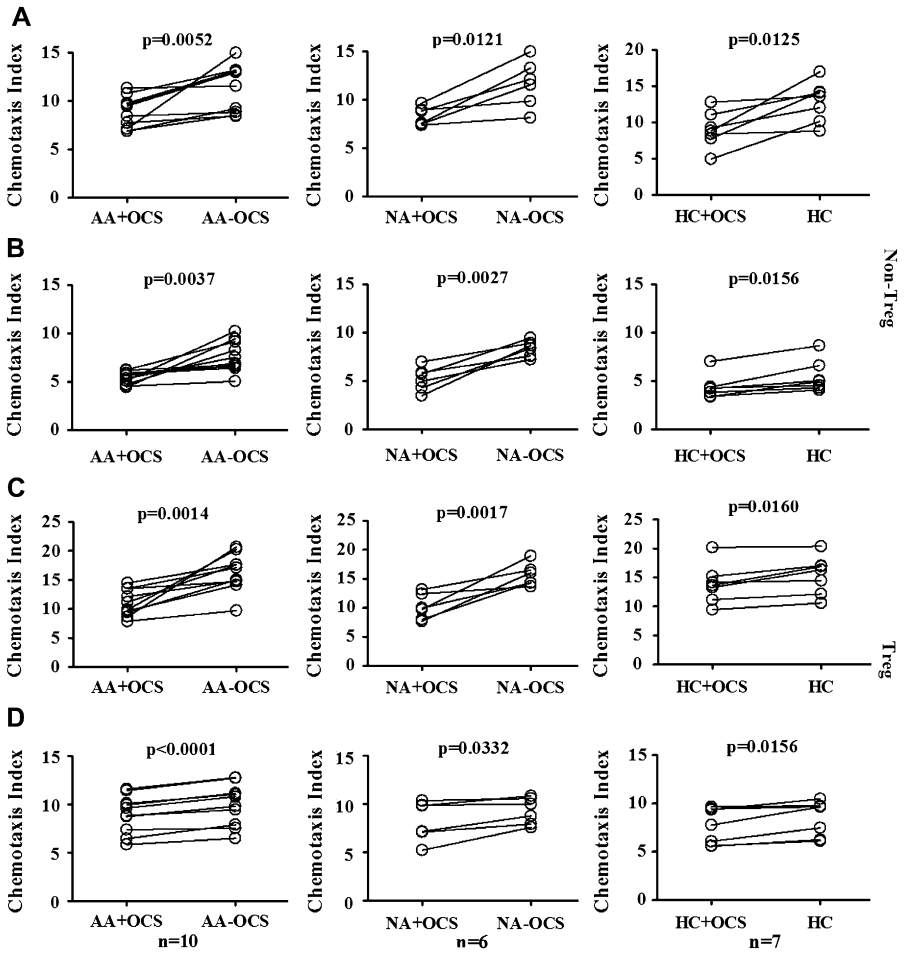

FIG E7. Effects of oral corticosteroids *(OCS)* on CCL17 and CCL22 chemotactic responses of Treg and non--Treg cells from AA, NA, and HC subjects. **A**, Chemotaxis assays of non-Treg cells toward CCL17. **B**, Chemotaxis assays of non-Treg cells toward CCL22. **C**, Chemotaxis assays of Treg cells toward CCL17. **D**, Chemotaxis assays of Treg cells toward CCL22. Comparisons were made between cells from the same AA (or NA) subjects collected with and without steroid withdrawal enforcement. HC cells were incubated with 1000 ng/mL OCS for 24 hours before being subjected to downstream assays. The Wilcoxon tests were used for the paired analyses of nonnormally distributed data (HC Treg and non-Treg cells in CCL22 chemotaxis assays). Paired *t* tests were used for all other analyses because the data were normally distributed.

TABLE E1. Subject demographics

Patient ID	Age (y)	Asthma duration (y)	Severity	Medication
AA1	56	15	Severe	Fluticasone, salmeterol: 500 mg twice daily
AA2	35	32	Severe	Fluticasone, salmeterol: 500 mg twice daily
AA3	37	34	Severe	Fluticasone, salmeterol: 500 mg twice daily
AA4	21	15	Severe	Fluticasone, salmeterol: 500 mg twice daily
AA5	18	17	Severe	Fluticasone, salmeterol: 500 mg twice daily
AA6	16	14	Severe	Fluticasone, salmeterol: 500 mg twice daily
AA7	18	16	Severe	Fluticasone, salmeterol: 250 mg twice daily
AA8	34	29	Moderate	Fluticasone, salmeterol: 250 mg twice daily
AA9	18	13	Moderate	Fluticasone, salmeterol: 250 mg twice daily
AA10	17	15	Moderate	Fluticasone, salmeterol: 250 mg twice daily
AA11	21	15	Moderate	Fluticasone, salmeterol: 250 mg twice daily
AA12	18	17	Moderate	Fluticasone, salmeterol: 250 mg twice daily
AA13	15	5	Mild	Albuterol puffs, 2/wk
AA14	21	6	Mild	Albuterol puffs, 2/wk
AA15	29	25	Mild	Albuterol puffs, 2/wk
AA16	29	2	Mild	Albuterol puffs, 2/wk
AA17	38	36	Mild	Albuterol puffs, 1.5/wk
AA18	18	17	Mild	Albuterol puffs, 1/wk
AA19	18	17	Mild	Albuterol puffs, 1/wk
AA20	15	5	Mild	Albuterol puffs, 1/wk
AA21	21	6	Mild	Albuterol puffs, 1/wk
AA22	21	6	Mild	Albuterol puffs, 1/wk
	24.27 ± 2.179	16.23 ± 2.07		
NA1	15	3	Severe	Fluticasone, salmeterol: 500 mg twice daily
NA2	28	23	Severe	Fluticasone, salmeterol: 500 mg twice daily
NA3	47	19	Severe	Fluticasone, salmeterol: 500 mg twice daily
NA4	22	9	Severe	Fluticasone, salmeterol: 500 mg twice daily
NA5	18	23	Severe	Fluticasone, salmeterol: 500 mg twice daily
NA6	25	5	Severe	Fluticasone, salmeterol: 500 mg twice daily
NA7	42	21	Moderate	Fluticasone, albuterol, puffs 1/wk
NA8	44	–	Moderate	Albuterol puffs, 2/wk
NA9	45	25	Mild	Albuterol puffs, 2/wk
NA10	55	–	Mild	Albuterol puffs, 2/wk
NA11	25	5	Mild	Albuterol puffs, 2/wk
NA12	19	–	Mild	Albuterol puffs, 1/wk
NA13	11	3	Mild	Albuterol puffs, 1/wk
NA14	15	13	Mild	Albuterol puffs, 1/wk
NA15	19	4	Mild	Albuterol puffs, 1/wk
NA16	35	15	Mild	Albuterol puffs, 1/wk
	29.06 ± 3.42	12.92 ± 2.37		

TABLE E2. Chemotactic index, phospho-AKT level, and clinical parameters of asthma of subjects

Patient ID	Comments	Allergic status	FEV_1	Chemotactic index	Phospho-AKT
AA1	Steroid withdrawal 12 h before second blood draw*	Tree, mold	52	1.429988159	3.193611
AA2	Steroid withdrawal 12 h before second blood draw*	Cat	53	1.609667123	3.366863
AA3	Steroid withdrawal 12 h before second blood draw*	Tree, dust mite	56	1.521250439	3.561444
AA4	Steroid withdrawal 12 h before second blood draw*	Tree	57	1.483614232	3.251166
AA5	Steroid withdrawal 12 h before second blood draw*	Dog	58	2.847170972	3.289645
AA6	Steroid withdrawal 12 h before blood draw	Mold	52	2.293563721	3.581024
AA7	Steroid withdrawal 12 h before blood draw	Tree, grass	55	2.302546396	4.988029
AA8	Steroid withdrawal 12 h before blood draw	Cat, mold	73	2.135721493	5.009265
AA9	Steroid withdrawal 12 h before blood draw	Weed	76	2.470334743	6.934832
AA10	Steroid withdrawal 12 h before blood draw	Weed, grass	73	2.496126877	7.268035
AA11	Steroid withdrawal 12 h before blood draw	Grass, dust mite	79	2.601930991	5.548491
AA12	Steroid withdrawal 12 h before blood draw	Tree, dust mite	67	1.860215054	5.223027
AA13	Steroid withdrawal 12 h before second blood draw*	Grass	92	3.278354633	7.607166
AA14	Steroid withdrawal 12 h before second blood draw*	Mold	85	3.866158656	7.754362
AA15	Steroid withdrawal 12 h before second blood draw*	Grass, mold	90	3.608086785	7.43685
AA16	Steroid withdrawal 12 h before second blood draw*	Grass	91	2.810528316	5.287654
AA17	Steroid withdrawal 12 h before second blood draw*	Tree, grass	93	3.407161926	7.364318
AA18	Steroid withdrawal 12 h before blood draw	Cat	95	2.937595129	7.828383
AA19	Steroid withdrawal 12 h before blood draw	Dog, dust mite	96	4.27696832	7.828383
AA20	Steroid withdrawal 12 h before blood draw	Weed, grass	92	5.168918919	9.211026
AA21	Steroid withdrawal 12 h before blood draw	Tree, cat	94	3.599328013	11.5698
AA22	Steroid withdrawal 12 h before blood draw	Tree, grass	95	2.920969832	9.775785
			76.09 ± 3.62	2.769 ± 0.21	6.222 ± 0.51
NA1	Steroid withdrawal 12 h before blood draw	None	51	4.751769975	10.81055747
NA2	Steroid withdrawal 12 h before blood draw	None	53	4.396421531	12.38808571
NA3	Steroid withdrawal 12 h before blood draw	None	55	5.292004049	16.55371966
NA4	Steroid withdrawal 12 h before blood draw	None	58	5.619329096	34.03530547
NA5	Steroid withdrawal 12 h before blood draw	None	59	4.505046315	30.92297959
NA6	Steroid withdrawal 12 h before blood draw	None	59	6.415036675	25.62070896
NA7	Steroid withdrawal 12 h before second blood draw*	None	69	4.508648039	30.92297959
NA8	Steroid withdrawal 12 h before second blood draw*	None	85	6.259968102	14.76721929
NA9	Steroid withdrawal 12 h before second blood draw*	None	85	5.936614467	11.86923077
NA10	Steroid withdrawal 12 h before second blood draw*	None	88	5.26615112	19.63818182
NA11	Steroid withdrawal 12 h before second blood draw*	None	95	7.620159348	30.92297959
NA12	Steroid withdrawal 12 h before second blood draw*	None	91	4.69254835	24.688
NA13	Steroid withdrawal 12 h before blood draw	None	88	4.297269841	16.14878882
NA14	Steroid withdrawal 12 h before blood draw	None	83	8.224229909	10.49745902
NA15	Steroid withdrawal 12 h before blood draw	None	95	5.533470296	24.73900826
NA16	Steroid withdrawal 12 h before blood draw	None	94	8.101769032	10.38476839
			75.5 ± 4.24	5.714 ± 0.33	20.31 ± 2.12

Summary data represent means and SDs.

*These subjects donated blood twice. Steroid withdrawal was not enforced in the first donation. Subjects did not have any changes in treatment between 2 blood donations. Chemotactic and AKT phosphorylation data represented results from experiments that were all performed with cells from subjects on steroid withdrawal.