HLA class I, Cw*01 and Cw*15 alleles can play a preventing role in serum IL-17 elevation associated with postmenopausal osteoporosis in Hungary

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BACKGROUND

IL-17 (named as IL-17A) is a novel family of inflammatory cytokines regulating neutrophil-recruitment and granulopoesis. IL-17 up-regulates the granulocyte colony-stimulating factor (G-CSF) therefore can accelerate osteoclastogenesis. Bone-wasting effect of IL-17 was demonstrated in multiple myeloma, periodontal bone loss and rheumatoid arthritis. TNF-α is a potent osteoclast maturating factor. Its secretion is stimulated by estrogen deficiency and its action can be potentiated with IL-17. HLA class I antigens are involved in cytotoxic immune responses binding to lymphocytes with CD8 molecules. HLA class II antigens are connected to lymphocytes expressing CD4 molecules.

The linkage between bone mineral density and immune system highlights an immunological aspect of osteoporosis. Trabecular bone loss is associated with estrogen deficiency and can be inhibited by addition of IL-17 monoclonal antibody.

PATIENTS AND METHODS

Sixty four postmenopausal women were divided into four subgroups according to BMDs and IL-17 levels. Subgroup OL: postmenopausal osteoporotic women with low IL-17 levels (2.85± 0.12 ng/ml), n=6, mean age of 60±6 years, mean lumbar BMD and T-score values: 0.695±0.05 g/cm² and -3.2±0.47; Subgroup OH: postmenopausal osteoporotic women with high IL-17 levels (3.79 ±0.55 ng/ml), n=35, mean age of 68±10 years, mean lumbar BMD and T-score values: 0.685±0.07 g/cm² and -3.31±0.63. Subgroup PL: postmenopausal osteopenic women with low IL-17 levels (2.9 ±0.12 ng/ml), n=8, mean age of 63±8 years, mean lumbar BMD and T-score values: 0.828±0.04 g/cm² and -2.01±0.47; Subgroup PH: postmenopausal osteopenic women with high IL-17 levels (3.48±0.38 ng/ml), n=15, mean age of 62±8 years, mean lumbar BMD and T-score values: 0.83±0.05 g/cm² and -1.98±0.47.

The HLA-A, -B, -Cw, -DRB1 and -DQB1 alleles were tested for all individuals with polymerase chain reaction (Biotest HLA PCR SSP kit, Biotest Bio-Rad Medical Diagnostics GmbH, Germany.

The serum IL-17A levels were measured with enzyme-linked immunosorbent assay (ELISA) (PeproTech, USA). The bone density (BMD g/cm²) of lumbar spines (L1-L4) was measured by dual energy X-ray absorptiometry (DXA) using Hologic Discovery Wi.

RESULTS

➤ The results using Hardy-Weinberg equilibrium (HWE) test, did not show remarkable difference among the subgroups (Table 1).

➤ The osteoporotic women with high IL-17 (subgroup OH) levels showed increased number of alleles in comparison with those found in subgroup with low IL-17 levels (OL) (Table 2).

➤ In women with high IL-17, the allele frequencies of HLA-Cw*01 and -Cw*15, as well as the haplotype frequencies of HLA-A*02—Cw*01 or HLA-A*02-DQB1*06, and HLA-Cw*01–DQB1*06 were significantly increased in osteopenic women compared with osteoporotic ones (Table 3 and 4).

➤ The pair of loci for HLA-Cw*01-DQB1*06 was not in linkage disequilibrium in subgroup OL (Table 5).

➤ The genetic distances between subgroups with high IL-17 (subgroups OH and PH) were closer together compared with subgroups with low IL-17 (OL and PL subgroups) (Figure 1).

HLA class I / HLA class II tables

Table 3: HLA class I and II allele frequencies (AF) in the subgroups of Hungarian postmenopausal women: OH: osteoporosis with high IL-17; OL: osteoporosis with low IL-17; PH: osteopenia with high IL-17; PL: osteopenia with low IL-17.

ᵃP<0.009, ᵇ P<0.02, ᶜP<0.037, ᵈP<0.031 and ᵉP<1.026 by Fischer's exact test between gray filled values. P<0.03 for HLA-Cw*15ᵈ, P<0.045 for HLA-DRB1*08 ᵈᵉ and P<0.008 for HLA-DQB1*06 ᵈᵉ by Fischer's exact test between osteoporotic (n=50) and osteopenic (n=14) women

Table 1 — Expected heterozygosity

	Osteoporosis		Osteopenia						
	low		high		low		high		
			IL-17 levels						
HLA-Loci	OL group	P value	OH group	P value	PL group	P value	PH group	P value	Mean	s.d.	Total of heterozygosity
A*	0.84848	1.0000	0.88613	0.5314	0.87500	0.9248	0.89888	0.9952	0.87712	0.02143	0.88374
B*	0.90970	1.0000	0.94741	0.8918	0.90833	1.0000	0.96092	1.0000	0.94659	0.02710	0.94783
Cw*	0.90909	0.5973	0.88549	0.7948	0.89833	1.0000	0.89046	0.2493	0.86334	0.04271	0.86626
DRB1*	0.88485	1.0000	0.88778	0.4327	0.90833	1.0000	0.89655	0.9385	0.91038	0.04445	0.89702
DQB1*	0.89394	1.0000	0.88535	0.3506	0.87500	0.9261	0.81830	0.5769	0.85967	0.03257	0.84781

Table 1: Expected heterozygosity and P values in the studied subgroups using Hardy-Weinberg equilibrium test for HLA-A, -B, -C, -DRB1 and -DQB1 loci. OH: osteoporosis with high IL-17, OL: osteoporosis with low IL-17; PH: osteopenia with high IL-17; PL: osteopenia with low IL-17.

Table 2 — Number of alleles

	Osteoporosis		Osteopenia				
	low	high	low	high			
			IL-17 levels				
HLA-loci	OL n=6	OH n=35	PL n=8	PH n=15	Mean	s.d.	Total
A*	8	15	8	12	10.750	3.403	17
B*	10	25	9	18	15.500	7.506	30
Cw*	7	10	6	10	8.250	2.062	12
DRB1*	9	11	9	9	10.000	1.155	13
DQB1*	7	7	5	6.750	1.258	9
Mean	8.600	13.800	7.800	10.800	10.250	2.685	16.200
s.d.	1.817	6.760	1.304	4.764	3.661	2.568	8.228

Table 2: Number of alleles at HLA-A, -B, -C, -DRB1 and -DQB1 loci in the studied subgroups. OH: osteoporosis with high IL-17, OL: osteoporosis with low IL-17; PH: osteopenia with high IL-17; PL: osteopenia with low IL-17.

Pairwise linkage disequilibrium — Table 5

HLA-loci	Exact P values in osteoporosis		Exact P values in osteopenia	
	Low IL-17 (OL) n=6	High IL-17 (OH) n=35	Low IL-17 (PL) n=8	High IL-17 (PH) n=15
A* and B*	0.31900	0.00000	0.03150	0.02464
A* and Cw*	0.04501	0.97588	0.54530	0.00000
B* and Cw*	1.00000	0.02009	0.22968	0.07774
A* and DRB1*	1.00000	0.21998	1.00000	0.14553
B* and DRB1*	1.00000	0.00000	0.70631	0.88511
Cw* and DRB1*	1.00000	0.71892	0.94672	0.28887
A* and DQB1*	0.00809	0.05882	0.21916	0.17248
B* and DQB1*	0.21316	0.00346	0.00652	0.61595
Cw* and DQB1*	0.51531	0.03292	0.98386	0.28673
DRB1* and DQB1*	1.00000	0.00415	0.15154	0.00009
A*02 and Cw*01	0.018			
A*02 and DQB1*06	0.018			
Cw*01 and DQB1*06	0.0778			
B*07 and DQB1*06				0.0009

Table 5: Pairwise linkage disequilibrium for all pairs of loci in the subgroups. OH: osteoporosis with high IL-17; OL: osteoporosis with low IL-17; PH: osteopenia with high IL-17; PL: osteopenia with low IL-17.
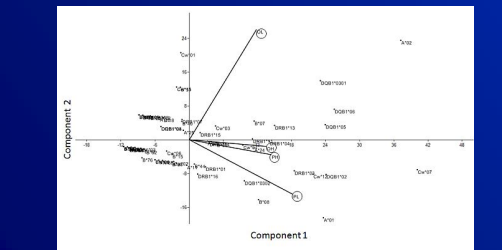
Table 4 — Haplotype frequencies

P<0.002 ᵃ, P<0.02 ᵇ, P<0.037 ᶜ and P<0.037 ᵈ by Fischer's exact test between gray filled values

Table 4: Haplotype frequencies (HF) in the subgroups of Hungarian postmenopausal women: OH: osteoporosis with high IL-17; OL: osteoporosis with low IL-17; PH: osteopenia with high IL-17; PL: osteopenia with low IL-17.

Figure 1: HLA-allele associations among the subgroups using principal correspondence analysis. OH: osteoporosis with high IL-17; OL: osteoporosis with low IL-17; PH: osteo-penia with high IL-17; PL: osteopenia with low IL-17, marked with open circles.

CONCLUSION

Our results revealed in postmenopausal osteoporosis that the high IL-17 levels, which cytokine is a bone-wasting accelerating factor, showed HLA associations at loci of HLA-A, -C and -DQB1. The absence of HLA-Cw*01 and -Cw*15 alleles, as well as HLA-A*02-Cw*01, -A*02-DQB1*06 and -Cw*01-DQB1*06 haplotypes was connected to high IL-17 levels. The HLA-allele association showed shorter genetic distances between subgroups with high IL-17 than between osteoporotic and osteopenic subgroups with low IL-17. In postmenopausal osteoporosis, the presence of HLA-Cw*01 and -Cw*15 alleles, as well as of HLA-A*02-Cw*01, -A*02-DQB1*06 and -Cw*01-DQB1*06 haplotypes demonstrated preventive role in the increase of IL-17.