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Section 4. Medical science

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POLYMORPHISM OF INTERLEUKIN 10 – ENCODING GENE AND ITS ROLE IN CYTOKINE RELEASE SYNDROME

Abstract. Relationship between polymorphism of cytokine-encoding genes and level of interleukin 10 in Cytokine release syndrome was researched. The algorithm of detection of predisposition of IL10 producing was conducted.

Keywords: Cytokine release syndrome, gene polymorphism, IL 10.

Introduction. Cytokine release syndrome (CRS) is caused by a large, rapid release of cytokines into the blood from immune cells affected by the immunotherapy [5, 56]. Cytokines are immune substances that have many different actions in the

body. Signs and symptoms of cytokine release syndrome include fever, nausea, headache, rash, rapid heartbeat, low blood pressure, and trouble breathing. Most patients have a mild reaction, but sometimes, the reaction may be severe or life threatening. Inter-

leukin 10 (IL10) is a one of the most important cytokines in human [3, 121]. The changing of cytokine level in organism can cause immunodeficiency and CRS. Detection of predisposition of IL10 producing in reaction of foreign antigen integration can help doctor in correct diagnosis and treatment choosing. It is more important for people with genetic inhering disease. The genetic aspects of IL10 producing are unknown [2, 149–50]. The knowledge about influence of gene variability on immune properties may explain the nature of some types of immune disorders such as CRS [5, 56].

The high level of IL10 normally must be 1.5 pg/mL. But it was possible to detect the possibility of IL10 producing only after foreign antigen [1, 554–7].

Correlation between the gene polymorphism and possibility of IL10 producing was still unknown. One is the most popular models of IL10 detection is analysis of patients' blood after infection by viruses [4, 323–335]. Nucleotide sequence of IL10-encoding gene was described [6, 37; 7, 4]. But the associated with DNA variability and possibility to IL10 producing was not described yet.

The aim of research is to detect relationship between polymorphism of cytokine-encoding genes and level of interleukin 10 in CRS and to conduct the algorithm of detection of predisposition of IL10 producing, if correlation between IL10 level and polymorphism of IL10-encoding gene is detected.

Material. 63 patients infected by Epstein-Barr virus were analyzed.

Methods: 1) Detection of interleukin 10 levels in 63 patients infected by Epstein-Barr virus (on the second day after the onset of symptoms) by ELISA test.

2) Identifying of interleukin 10 levels in 63 patients infected by Epstein-Barr virus after convalescence by (ELISA).test.

3) Development of primers and temperature-time conditions for polymorphic regions of cytokine-encoding genes by VECTORNTI11 program.

4) Detection of polymorphism of cytokine-encoding genes in 63 patients by polymerase chain reaction (PCR).

5) Identifying of relationship between polymorphism of cytokine-encoding genes and level of interleukin 10 by Spearman rank correlation coefficient.

6) Test and evaluation will be provided on DNA sequences of human cytokine-encoding genes obtained from National Centre of Biotechnological Information by VECTORNTI11 program [8].

7) In case if statistical error is more than 30%, relationship will be defined as insignificant.

Results. In order to detect the effect of allele combination on the possibility to produce IL10 there were used statistics methods.

Each allele was encoded by the following Latin symbols: 512 bp – G; 521 bp – S; 530 – Y; 340 bp – K; 666 bp – R; 672 bp – D; 688 bp – C; 690 bp – Q (tab. 1).

Table 1. – Allele condition of examined patients

Number of patient	Product of PR ₁ primers, (base pair)	Product of PR ₂ primers, (base pair)	Product of PR ₃ primers, (base pair)	Product of PR ₄ primers, (base pair)
1	2	3	4	5
1N*	R, R	C, Q	Y, Y	G, G
2N	D, D	C, C	Y, Y	G, S
3H**	R, D	C, Q	Y, K	G, S
4N	R, D	C, C	Y, Y	G, S
5N	R, R	C, C	Y, Y	G, G
6N	R, R	C, Q	Y, Y	G, S
7N	R, R	C, C	Y, Y	G, S
8N	R, R	C, C	Y, Y	G, S

1	2	3	4	5
9N	R, R	C, C	Y, Y	G, S
10N	R, D	C, C	Y, Y	G, S
11N	D, D	C, Q	Y, Y	G, S
12N	R, R	C, C	Y, Y	G, G
13N	R, R	C, C	Y, Y	G, S
14H	R, D	C, Q	Y, Y	G, S
15N	D, D	C, C	Y, Y	G, S
16N	D, D	C, C	Y, Y	G, S
17N	R, D	C, Q	Y, Y	G, G
18N	R, R	C, C	Y, Y	G, S
19N	R, D	C, C	Y, Y	G, S
20N	R, D	C, Q	Y, Y	G, G
21N	R, D	C, Q	Y, Y	G, S
22N	R, R	C, C	Y, Y	G, G
23N	R, D	C, C	Y, Y	G, S
24H	R, R	C, C	Y, K	G, S
25N	R, D	C, C	Y, Y	G, S
26N	R, R	C, C	Y, Y	G, S
27N	R, R	C, C	Y, Y	G, S
28N	R, R	C, C	Y, Y	G, S
29N	R, R	C, C	Y, Y	G, S
30H	R, R	C, C	Y, K	G, G
31N	R, R	C, Q	Y, Y	G, S
32N	R, R	C, C	Y, Y	G, S
33H	R, D	C, C	K, K	G, S
34N	D, D	C, Q	Y, Y	G, S
35N	R, R	C, C	Y, Y	G, S
36N	R, R	C, Q	Y, Y	G, S
37H	D, D	C, Q	Y, K	G, S
38N	R, R	C, C	Y, Y	G, S
39N	R, R	C, C	Y, Y	G, S
40N	R, D	C, C	Y, Y	G, S
41N	R, R	C, C	Y, Y	G, S
42N	R, R	C, Q	Y, Y	G, S
43N	R, R	C, C	Y, Y	G, S
44H	D, D	C, Q	K, K	G, G
45N	R, R	C, C	Y, Y	G, S
46N	R, R	C, Q	Y, Y	G, S
47N	D, D	C, C	Y, Y	G, S
48N	R, D	C, Q	Y, Y	G, S

1	2	3	4	5
49H	R, R	C, C	Y, K	G, S
50N	R, R	C, C	Y, Y	G, S
51H	D, D	C, Q	Y, K	G, S
52N	R, R	C, C	Y, Y	G, S
53N	R, R	C, C	Y, Y	G, S
54N	R, D	C, Q	Y, Y	G, G
55N	D, D	C, Q	Y, Y	G, S
56N	R, R	C, C	Y, Y	G, G
57H	R, R	C, Q	Y, K	G, S
58N	R, R	C, C	Y, Y	G, S
59H	R, D	C, Q	K, K	G, S
60N	D, D	C, Q	Y, Y	G, S
61N	R, R	C, Q	Y, Y	G, S
62N	R, D	C, C	Y, Y	G, S
63N	R, D	C, C	Y, Y	G, S

* H – level of interleukin 10 is > 1.5 pkg/ml.

** N – level of interleukin 10 is < 1.5 pkg/ml.

Results of IL10 detection are present in (tab. 2)

Table 2. – Results of ELISA test

Number of patient	Difference of levels of interleukin 10 after the onset of symptoms and after convalescence (pkg/ml)
1	2
1	0.9
2	1.3
3	2.5
4	1.0
5	1.4
6	1.3
7	1.4
8	1.4
9	1.2
10	1.0
11	0.8
12	1.4
13	1.2
14	1.7
15	1.1
16	1.3

1	2
17	0.9
18	1.4
19	1.1
20	1.3
21	1.3
22	1.1
23	1.2
24	2.8
25	1.3
26	1.2
27	1.0
28	1.3
29	0.7
30	2.6
31	1.2
32	1.3
33	3.1
34	0.9
35	1.3
36	1.2

1	2
37	2.3
38	1.2
39	1.1
40	1.3
41	1.0
42	0.8
43	1.1
44	4.0
45	1.2
46	1.4
47	0.7
48	1.1
49	2.1
50	1.4
51	1.9
52	1.2
53	1.3
54	1.3
55	0.8
56	1.1
57	2.6
58	1.3
59	4.1
60	1.2
61	1.3
62	1.2
63	1.0

Identifying of relationship between polymorphism of cytokine-encoding genes and level of interleukin 10 was provided by Spearman rank correlation coefficient. There were two rank parameters:

high tendency to release interleukin 10 (if difference of levels of interleukin 10 after the onset of symptoms and after convalescence is 1.5 pkg/ml or more) and low tendency to release interleukin 10 (if difference of levels of interleukin 10 after the onset of symptoms and after convalescence is less than 1.5 pkg/ml). As result of statistical analysis there was detected that Spearman's rank correlation coefficient was $p = 0.881$.

Critical point $T_{cr} = 0.190$.

$|p| > T_{cr}$ – null hypothesis is not confirmed, rank correlation between traits is significant.

Critical point of the bilateral critical region $t(\alpha, k) = 1.734$.

Confidence interval $r = (0.79; 0.97)$

Error 18%

Conclusion. Therefore, the relationship between levels of IL10 after the onset of symptoms and after convalescence, and polymorphism of cytokine-encoding genes is straight forward, significant and within the confidence interval. The detection of IL10 in human is very important as it possesses high anti-inflammatory properties which play a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis. It is known that dysregulation of IL10 is associated with enhanced immunopathology in response to infection as well as increased risk for development of many autoimmune diseases. Thus a fundamental understanding of IL10 gene expression is critical for our comprehension of disease progression and resolution of host inflammatory response.

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