JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1985, p. 318–319
0095-1137/85/080318-02$02.00/0

Vol. 22, No. 2

Interleukin 2 Production in Whole Blood Culture: a Rapid Test of Immunity to *Francisella tularensis*

RIITTA KARTTUNEN,[1]* JORMA ILONEN,[2] AND ELJA HERVA[2]

Department of Medical Microbiology, University of Oulu, SF-90220 Oulu 22,[1] and National Public Health Institute, SF-90100 Oulu 10,[2] Finland*

Received 22 February 1985/Accepted 14 May 1985

The measurement of interleukin 2 from antigen-stimulated whole blood culture supernatants was used to detect cell-mediated immunity in subjects sensitized against *Francisella tularensis*. The amount of interleukin 2 produced differentiated a positive immune reaction sensitively and reliably in a 24-h culture. Whole blood culturing is an easy method for producing interleukin 2.

Natural infection with *Francisella tularensis* and vaccination cause strong cell-mediated immunity, which can be detected with the delayed-type skin test (2) or the in vitro lymphocyte stimulation test, with either separated lymphocytes or whole blood (a modification) (6, 8). The lymphoblast transformation (LBT) response starts to become positive at the end of the first week and is positive in 96.8% of cases by 14 days after the onset of symptoms, when only 53.3% of patients have antibodies, as measured by the agglutination method (7). Thus, in many cases a diagnosis can be achieved earlier by testing for the LBT response. One drawback of the LBT test is the time it takes (7 days).

The purpose of this study was to compare the production of interleukin 2 (IL2) with the LBT response to see if a positive reaction could be identified earlier by this method, as it has been shown with herpes simplex virus antigen that the production of IL2 correlates with the LBT response as a measure of sensitization (1, 4). The purpose was also to test whether IL2 could be produced by the convenient method of whole blood culturing and whether it is measurable by the IL2 assay used here.

The subjects were 7 persons vaccinated against *F. tularensis* in 1979 to 1980 and 14 laboratory personnel (controls) with no anamnesis of a natural infection or vaccination. The LBT response was measured as described earlier (7) by pipetting 50 μl of whole blood diluted 1:4 in RPMI 1640 medium containing 10% inactivated human serum, antibiotics, glutamine, 50 μl of antigen (whole Formalin-killed *F. tularensis*; National Bacteriological Laboratory, Stockholm, Sweden), and 100 μl of medium onto a microtiter plate. The final antigen concentration was 150 μg/ml, and the incubation time was 7 days, [^{3}H]thymidine being present for the last 24 h. The IL2 supernatants were obtained in tubes with 1.2 ml of whole blood plus 0.4 ml of antigen by incubation for 24 or 48 h. After centrifugation the supernatants were harvested and stored at −20°C until tested. Test cells for the IL2 assay were obtained by stimulating mononuclear cells from a healthy person with concanavalin A for 5 days (5, 9). A 100-μl portion of each supernatant dilution was added to 10^4 test cells on a microtiter plate and incubated for 48 h, [^{3}H]thymidine being present for the last 24 h. Counts at a final supernatant dilution of 1:2 were used for the results, as these always lay on the decreasing slope of the dilution curve. The control counts of the test cells were 270 with *F. tularensis* antigen and 121 with medium. The counts with antigen have not been subtracted from the values given in Table 1. The LBT response and IL2 production results are shown in Table 1. Counts of more than 1,000 were used as a limit for the positive LBT response, based on studies done earlier with 211 control persons (7). Five of the seven vaccinated subjects had a positive LBT response, and these same five persons (subjects 1 through 5) also produced IL2 to a degree that clearly differentiated them from the control subjects. The remaining two vaccinated subjects (subjects 6 and 7) had a negative LBT response, but even they produced IL2 over the 95% confidence interval of the control subjects. The IL2 counts for control subjects other than 1 and 2 did

TABLE 1. *F. tularensis* antigen-induced lymphoblast transformation and IL2 production in vaccinated and control subjects

Subject	Antigen-induced LBT response (cpm)	IL2 production (cpm) at[a]:	
		24 h	48 h
Vaccinated			
1	32,053	7,794	7,257
2	31,323	3,606	3,582
3	5,517	3,580	1,180
4	4,385	1,374	1,210
5	2,766	2,090	1,704
6	735	928	1,114
7	85	938	604
Control			
1	122	512	430
2	107	367	339
3	50	137	57
4	28	59	45
5	127	79	55
6	342	173	225
7	354	155	89
8	82	156	145
9	145	129	129
10	536	217	96
11	20	91	96
12	15	122	129
13	196	44	67
14	12	127	157

[a] For control subjects, the mean values at 24 and 48 h were 169 and 147 cpm, respectively, and the mean ± 2 standard deviation values at 24 and 48 h were 422 and 373 cpm, respectively.

* Corresponding author.

not exceed those for the test cells with antigen. The amount of IL2 produced by the vaccinated subjects and the negative controls differed after incubations of both 24 and 48 h ($P < 0.001$, Mann Whitney U-test).

It has been shown here that the measurement of IL2 can be used to differentiate a positive cell-mediated immune reaction to *F. tularensis* and may even be a more sensitive way of detecting a memory for *F. tularensis* antigen than the standard LBT response, as two of the vaccinated subjects with no LBT response did show some IL2 production. The LBT response test was repeated three times on one of them (subject 6), always with a negative result (data not shown). As a positive immune reaction of vaccinated subjects to *F. tularensis* could be detected by measuring IL2, this method should also be tried on patients with recent infections. A rapid enzyme immunoassay for IL2 measurement, as recently described (3), will be of use in shortening assay time.

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