INFECTION AND IMMUNITY, June 1991, p. 1948–1953
0019-9567/91/061948-06$02.00/0

Vol. 59, No. 6

Development of *Francisella tularensis* Antigen Responses Measured as T-Lymphocyte Proliferation and Cytokine Production (Tumor Necrosis Factor Alpha, Gamma Interferon, and Interleukin-2 and -4) during Human Tularemia

H.-M. SURCEL,[1]* H. SYRJÄLÄ,[1] R. KARTTUNEN,[2] S. TAPANINAHO,[1] AND E. HERVA[1]

National Public Health Institute[1] and Department of Medical Microbiology, University of Oulu,[2] 90100 Oulu, Finland

Received 9 October 1990/Accepted 28 February 1991

The lymphocyte immune reactivity of 12 tularemia patients to *Francisella tularensis* antigens prepared from the bacterial cell envelope was examined during a 14-week follow-up study. Lymphocyte blast transformation responses of peripheral blood mononuclear cells (PBMC) to different protein antigens appeared simultaneously 2 weeks after the first symptoms of tularemia, indicating that none of these antigens had any special role at the early phase of immunization. While the lymphocyte blast transformation responses of total lymphocytes to all bacterial antigens were negative in the week 1 samples, continuously growing *F. tularensis*-specific T-lymphocyte lines were obtained from PBMC at the same time, indicating that an immune response had already occurred. Later, the T-lymphocyte lines and lymphocyte blast transformation responses were similar. Lymphocyte activation among the PBMC was reflected in an increased number of HLA-DR antigen-expressing, CD4-positive T lymphocytes ($CD4^+$ DR^+). The mean secretion of soluble CD8 from *F. tularemia* antigen-stimulated PBMC increased 2 weeks after tularemia onset, but the mean number of $CD8^+$ DR^+ T lymphocytes did not vary during the study period and no correlation could be found between the soluble CD8 and number of $CD8^+$ DR^+ T lymphocytes. *F. tularemia* antigen-induced cytokine production was measured from the PBMC supernatants. High levels of tumor necrosis factor alpha were detected from the first week onwards. The highest levels of interleukin-2 and gamma interferon were recorded during the second and third weeks, respectively, after tularemia onset. Interleukin-4 could not be demonstrated in the lymphocyte supernatants.

Tularemia, which is caused by a gram-negative bacterium, *Francisella tularensis*, induces cell-mediated immunity that resembles immune responses to other intracellular bacteria such as *Mycobacterium tuberculosis*, *Mycobacterium leprae*, or *Listeria monocytogenes*. Tularemia memory immunity is reflected in lymphocyte blast transformation (LBT) responses to numerous bacterial proteins separated from the cell envelope (12, 14, 17). LBT responses to whole bacterial antigen develop in 2 weeks (7, 18) and are considered a highly specific marker of tularemia immunity (7, 21, 22). Whether the immune responses to separated protein antigens are developed simultaneously is not known.

Gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α) have been found essential for host resistance to mycobacterial infections (3), *L. monocytogenes* (8, 11), *Legionella pneumophila* (12), and also *F. tularensis* (1). Cytokine production, such as secretion of IFN-γ or interleukin-2 (IL-2), can also be used for identification of specific cellular immunity (5, 16).

The purpose of this study was to analyze the development of LBT responses to different protein antigens prepared from *F. tularensis*, the activation of $CD4^+$ and $CD8^+$ lymphocytes, and the appearance of in vitro antigen-induced cytokine (TNF-α, IL-2, IFN-γ, and IL-4) secretion.

MATERIALS AND METHODS

Patients. The study group consisted of six men and six women (median age, 46 years; range, 32 to 74 years) who had ulceroglandular tularemia (ten cases) or pulmonary tularemia (two cases). The diagnosis of tularemia was confirmed serologically. The agglutination antibody titer changed from negative (<1:40) to at least 1:320 in 11 patients and from 1:80 to 1:280 in 1 patient.

The patients with ulceroglandular and pulmonary tularemia were analyzed together because we had found earlier that the clinical picture and severity of tularemia did not cause any difference in the strength of the lymphocyte response (18). Nine patients received ciprofloxacin, 750 mg twice daily for 10 days; one patient received norfloxacin, 400 mg twice daily for 10 days; one patient received doxycycline, 150 mg once a day for 10 days; and the last patient received doxycycline first and ciprofloxacin later. In our experience, the fever usually vanishes within a few days of starting the fluoroquinolone therapy.

The time from the onset of tularemia to the first visit to the health care center varied among patients from 3 to 21 days. Blood specimens were taken an average of 7 days after the beginning of symptoms, at intervals of 7 days for 6 weeks, and then 14 weeks later. Because the results were adjusted according to days from the beginning of the symptoms, the number of specimens in the experiments varied.

Antigen preparations. *F. tularensis* LVS (BB IND 157.6111) was supplied by the U.S. Army Medical Research Institute for Infectious Diseases, Fort Detrick, Md., and was cultured on medium consisting of 1.5% (wt/vol) proteose peptone no. 3 (Difco Laboratories, Detroit, Mich.), 55.5 mM glucose, 85.5 mM NaCl, and 0.2 mM $FeSO_4 \cdot 7H_2O$ in 54 mM K_2HPO_4 buffer (pH 7.4) supplemented with IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.). The bacteria were harvested at the early logarithmic growth phase by centrifugation, killed by treatment with 0.1% formalde-

* Corresponding author.

TABLE 1. PBMC responses to FT-Ag antigen (1 μg/ml) and 17- and 40-kDa proteins (2 μg/ml)

Wk after first tularemia symptoms	PBMC response (cpm) to:											
	Buffer			FT-Ag			17-kDa protein			40-kDa protein		
	Median	Range	n	Median	Range	n	Median	Range	n	Median	Range	n
1	88	37–202	6	187.5	24–1,419	6	195	96–791	3	118	95–588	3
2	163	45–1,415	10	11,936[a]	25–61,357	10	4,877[a]	185–19,129	7	483	149–2,464	7
3	137	55–710	9	29,242[b]	412–57,092	9	5,822[b]	132–17,466	9	501[c]	149–3,422	9
4	165	47–339	11	23,843[b]	3,661–37,981	11	9,054[b]	639–34,850	10	230[c]	98–7,800	10
5	216	78–874	9	23,294[b]	11,413–26,779	9	7,277[b]	335–13,490	8	754	83–4,064	8
14	354	168–958	9	26,457[b]	3,207–36,728	9	3,939[b]	929–15,680	3	89	61–632	3

[a] $P < 0.025$.
[b] $P < 0.001$.
[c] $P < 0.05$.

hyde, and washed with physiological phosphate buffer (Dulbecco phosphate-buffered saline [PBS]; GIBCO, Paisley, United Kingdom). The bacteria thus prepared were suspended in PBS at the optimal concentration for use as a whole-cell *F. tularensis* antigen (FT-Ag) in the LBT test (7).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) fractions were prepared from *F. tularensis* total envelope material by preparative SDS-PAGE (15).

The heat-modifiable membrane proteins with apparent molecular masses of 17 and 40 kDa were purified by preparative SDS-PAGE as described earlier (17). Briefly, a protein band to be purified was excised horizontally from the gel, and the protein was eluted from the gel by shaking overnight at room temperature with 15 ml of 0.1% (wt/vol) SDS supplemented with 1 mM phenylmethylsulfate (Sigma Chemical Co., St. Louis, Mo.). After elution, the protein was concentrated to about 500 μl, using Immersible CX-10 ultrafiltration units (Millipore Corp., Bedford, Mass.). The resulting protein was reanalyzed by SDS-PAGE and was either purified further or diluted to be used as antigen in the LBT test.

Mononuclear cell cultures. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Lymphoprep gradient centrifugation (Nyegaard & Co. a/s, Oslo, Norway) and suspended in RPMI 1640 (GIBCO) supplemented with 10% human AB serum. The LBT responses were studied as described previously (7). Briefly, 50,000 isolated PBMC were cultured in triplicate wells on 96-well round-bottom microtiter plates in the presence of antigen in a total volume of 200 μl for 6 days in a humidified 5% CO_2 atmosphere at 37°C. [^{3}H]thymidine was present in the cultures for the last 18 h of the incubation, and proliferation was assayed from the incorporated radioactivity measured with a liquid scintillation counter (LKB Wallac, Turku, Finland). The results are expressed as mean counts per minute minus background of triplicate cultures. The minimum amounts of antigens giving an efficient lymphocyte response were used for FT-Ag (1.0 μg of protein per ml), the SDS-PAGE fractions (2.0 μg/ml), and the purified membrane proteins (2 μg/ml). The concentration of purified proteins was determined after the lymphocyte responses were studied to be detectable at protein concentrations of 1.0 to 10.0 μg/ml (17). Tetanus toxoid (10 μg/ml) was used as a control antigen in each experiment.

TABLE 2. Median LBT responses of FT-Ag-specific TLL obtained from PBMC of eight patients 1, 2, and 3 weeks after the first tularemia symptoms

TLL induced at wk:	LBT response [mean cpm (range)] to:		
	FT-Ag	17-kDa protein	40-kDa protein
1	4,525 (956–8,733)	1,211 (538–9,008)	1,818 (229–8,561)
2	8,117 (4,677–12,745)	6,934 (484–11,393)	4,346 (0–8,061)
3	6,742 (328–13,205)	4,384 (431–8,210)	3,611 (661–9,286)

[a] Data are expressed as means, minus background (<250 cpm), of triplicate cultures after 2 days of incubation with FT-Ag (1 μg of protein per ml) or the 17- or 40-kDa protein (2 μg/ml) and autologous antigen-presenting cells.

F. tularensis-specific T-cell lines (TLL) were induced with FT-Ag as described earlier (16). After 7 days of FT-Ag stimulation, the live lymphocytes were collected and the surface phenotype of the activated cells was analyzed by immunofluorescence. The residual lymphocytes were restimulated with FT-Ag and fresh irradiated autologous PBMC (10^6/ml) as the antigen-presenting cells in AB medium supplemented with 20 U of recombinant IL-2 (Janssen Biochimica, The Netherlands) per ml, as described earlier (16). The restimulation was repeated five times for periods of 7 days over 6 weeks, and the continuously growing TLL were tested for antigen specificity.

Cytokine measurement. Cytokine production was induced by incubating the isolated PBMC (10^6/ml) without antigen or with FT-Ag (1.0 μg/ml) in a total volume of 1 ml in a humidified 5% CO_2 atmosphere at 37°C. By previously established kinetics of IL-2 and IFN-γ production, supernatants for maximal secretion of IL-2 induced by FT-Ag were collected at 2 days and those of IFN-γ were collected at 5 days (5). Since TNF-α production is known to be influenced by IFN-γ (23), the supernatants for TNF-α were collected at day 5. Troye-Blomberg et al. (24) have demonstrated mRNA in human cells 24 h after antigen stimulation; thus, the supernatant for IL-4 analysis was collected at day 2. The supernatants were collected by centrifugation and kept at −70°C until analysis.

IL-2 was measured by using an IL-2-dependent mouse cytotoxic T-cell line (kindly donated by Toini Berzin, University of Stockholm), as described earlier by Surcel et al. (16).

The levels of soluble CD8 (T Cell Sciences, Cambridge, Mass.), IL-4 (Genzyme Corp., Boston, Mass.), TNF-α (Endogen Inc., Boston, Mass.), and IFN-γ (Endogen) were measured by enzyme-linked immunosorbent assay (ELISA), using reagents and directions supplied by the manufacturer.

HLA-DR expression of stimulated cells. T-cell activation was characterized by analyzing the HLA-DR antigen on the surface of CD4-positive (T helper) or CD8-positive (T

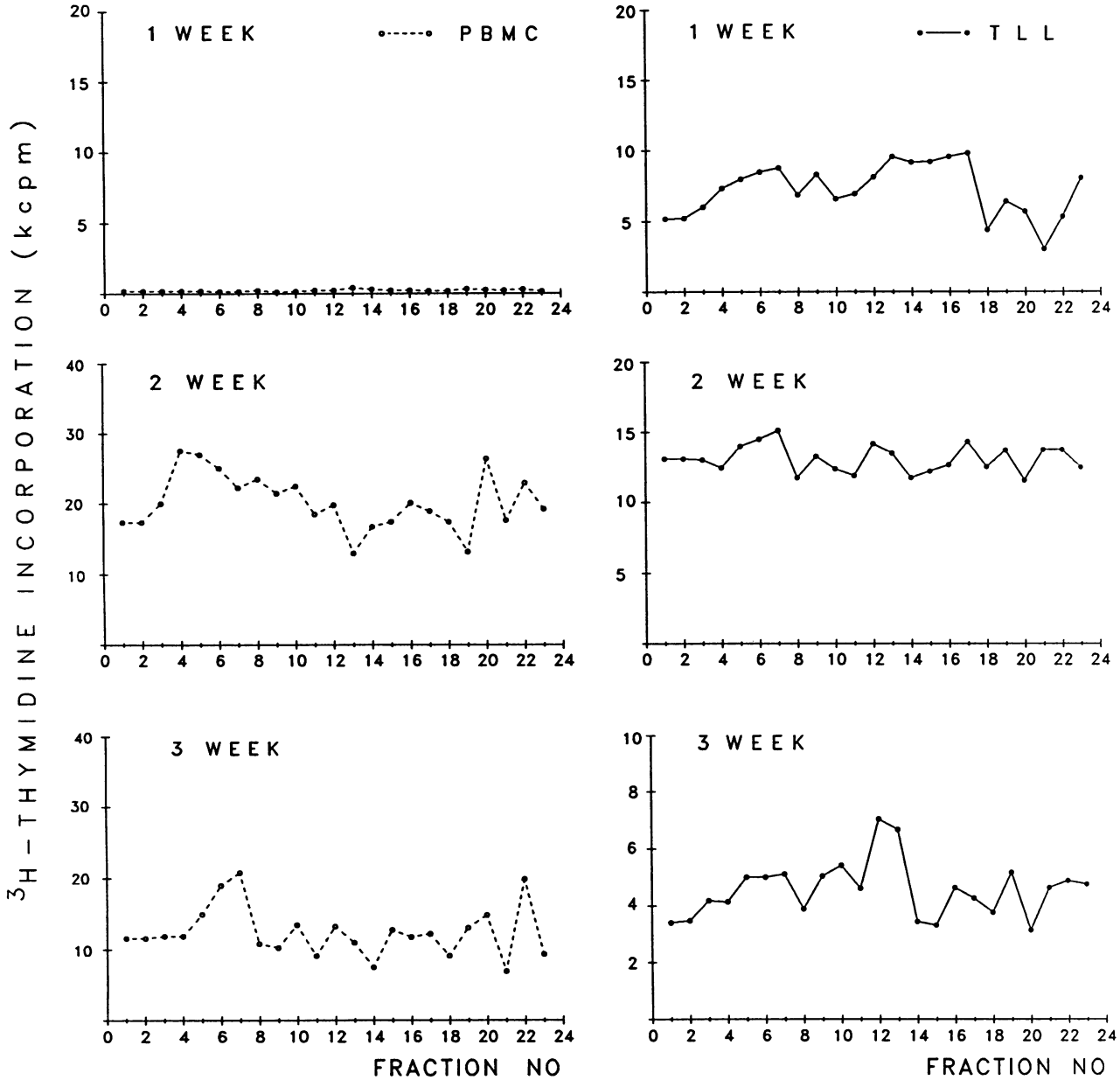

FIG. 1. Proliferative responses of PBMC (○) from one patient 1, 2, and 3 weeks after the onset of tularemia symptoms and corresponding FT-Ag-specific TLL (●) to SDS-PAGE fractions (no. 1 to 23) prepared from *F. tularensis* total envelope. Data are expressed as mean counts per minute obtained from triplicate cultures minus background (<250 cpm) for PBMC after 6 days of incubation with antigen (2 μg of protein per ml) and for TLL after 2 days of incubation with antigen and antigen-presenting cells. The mean LBT response to the SDS-PAGE fractions ± standard error for PBMC was 357 ± 69 cpm in week 1, 4,284 ± 816 cpm in week 2, and 2,428 ± 314 cpm in week 3; that for TLL was 645 ± 124 cpm in week 1, 651 ± 64 cpm in week 2, and 358 ± 53 cpm in week 3.

suppressor/cytotoxic) T cells of 6-day FT-Ag-stimulated PBMC, using the double immunofluorescence assay. A total of 0.4×10^6 stimulated cells were labeled with monoclonal antibodies (20 μl/test) conjugated with different fluorochromes (phycoerythrin or fluorescein-conjugated anti-Leu2, Leu3, HLA-DR antibodies; Becton Dickinson, Mountain View, Calif.), using directions supplied by the manufacturer. Some 5,000 labeled cells were analyzed by flow cytometry (laser power, 15 mW; FACS analyzer; Becton Dickinson), using forward light scatter to exclude dead cells.

Statistics. The antigen-stimulated PBMC responses were compared with the nonstimulated responses by Mann-Whitney analysis.

RESULTS

LBT responses induced by *F. tularensis* antigens. The separated PBMC were stimulated with FT-Ag, heat-modifiable membrane proteins with molecular masses of 17 kDa (contained in unpurified form in SDS-PAGE fraction 1) and 40 kDa (contained in unpurified form in SDS-PAGE fraction

TABLE 3. Number of $CD4^+$ and $CD8^+$ lymphocytes expressing HLA-DR antigen as an activation marker

Wk after first tularemia symptoms	n	% of FT-Ag-stimulated PBMC (mean)				
		$CD4^+$	$CD4^+$ DR^+	$CD8^+$	$CD8^+$ DR^+	$CD4^+$ DR^+/ $CD8^+$ DR^+
1	5	52.0 ± 16.1	4.8 ± 1.9	30.4 ± 8.8	2.5 ± 1.2	4.4 ± 3.3
2	8	62.1 ± 21.8	34.5 ± 10.5[a]	14.0 ± 7.9	4.4 ± 1.3	13.3 ± 3.6
3	9	63.7 ± 22.2	45.3 ± 9.1[b]	14.6 ± 11.0	8.5 ± 3.1	23.3 ± 12.8
4	4	63.5 ± 31.2	45.3 ± 15.1[b]	5.83 ± 1.9	4.6 ± 0.7	10.7 ± 3.5
14	8	44.4 ± 9.7	12.5 ± 4.0[a]	21.3 ± 7.2	5.2 ± 0.7	2.8 ± 1.2

[a] $P < 0.01$.
[b] $P < 0.001$.

14), and SDS-PAGE fractions. Table 1 shows that a positive LBT response to FT-Ag or to the 17-kDa protein appeared 2 weeks after the first symptoms of tularemia ($P < 0.025$). The positive LBT response to tetanus toxoid (>500 cpm) showed that the PBMC separated 1 week after the onset of tularemia were functional (data not shown). The median LBT response to the 40-kDa protein, on the other hand, did not differ significantly from the nonstimulated level. However, the lymphocytes from seven patients were occasionally stimulated effectively by the 40-kDa protein 3 to 5 weeks after the onset of tularemia, which is seen as a wide range for the LBT response against this protein (Table 1). The LBT responses to the SDS-PAGE fractions appeared simultaneously within 2 weeks of the symptoms of tularemia.

The PBMC in specimens taken 1, 2, and 3 weeks following tularemia onset were cultured to FT-Ag-specific TLL. The antigen specificity of the continuously growing TLL was confirmed after 6 weeks by nonresponsiveness to tetanus toxoid (<500 cpm) and by an LBT response to FT-Ag (>2,500 cpm). The TLL responses against the SDS-PAGE fractions resembled those of the corresponding fresh PBMC (data shown for one patient in Fig. 1). Figure 1 (week 1) shows an example of tularemia-specific TLL successfully obtained from the first-week samples of five of eight patients whose LBT responses to FT-Ag were still negative ($P > 0.1$). Of the 24 TLL, 14 also responded to the 40-kDa envelope protein ($P < 0.001$; Table 2).

HLA-DR expression of stimulated lymphocytes and soluble CD8. The positive LBT response to FT-Ag was also detected in stimulated PBMC in vitro by an increased number of activated T-helper ($CD4^+$ DR^+) lymphocytes in the second-week specimens (Table 3), whereas the mean values of activated suppressor or cytotoxic T lymphocytes ($CD8^+$ DR^+) did not change during the study period.

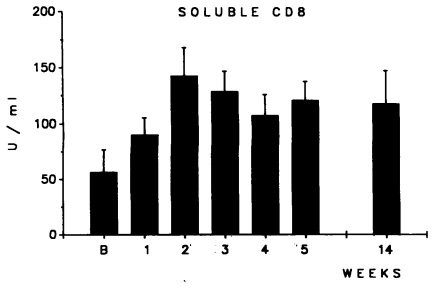

FIG. 2. Mean levels of soluble CD8 in FT-Ag-stimulated PBMC supernatants in weeks 1 to 5 and 14 after the first tularemia symptoms (thin bars show standard errors). The levels of soluble CD8 differed significantly from the nonstimulated background (B) levels in weeks 2, $P < 0.01$, and 3, $P < 0.001$.

Soluble CD8 was measured from antigen-stimulated PBMC supernatants as another marker for $CD8^+$ lymphocyte activation. The FT-Ag induced a significant increase of soluble CD8 in the PBMC supernatants 2 and 3 weeks after the onset of tularemia and remained elevated, though not significantly, for up to 14 weeks (Fig. 2).

Cytokine secretion. Figure 3 shows the time courses of the changes in IFN-γ, TNF-α, and IL-2 secretion by PBMC stimulated with FT-Ag. TNF-α was secreted at high levels 1 week after the first tularemia symptoms. The highest level of IL-2 was found in the second week after the first symptoms of tularemia, and maximum IFN-γ was measured 3 weeks after the first tularemia symptoms. IL-4 was not found in the lymphocyte supernatants above the background level (0.01 ng/ml) (results not shown).

DISCUSSION

Our results show that the immune responses to different envelope SDS-PAGE fractions prepared from the total envelope proteins develop simultaneously within 2 weeks of *F. tularensis* infection. Thus, none of the numerous *F. tularensis* envelope proteins which were earlier shown to act as T-cell-stimulating antigens in tularemia memory immunity (13, 15) plays any special role in the early development of infection immunity.

The present results also show that the 40-kDa outer membrane protein of *F. tularensis* effectively stimulated lymphocytes of some patients between 2 and 4 weeks after the onset of tularemia symptoms. In our previous study the heat-modifiable 40-kDa protein did not induce detectable lymphocyte proliferation in *F. tularensis* vaccine-immunized subjects even though the protein concentration was increased and the other envelope proteins purified simultaneously were stimulative (17), leading us to assume that the 40-kDa protein possessed few or no T-cell epitopes. Thus, the present data support the former assumption, because a positive LBT response to the 40-kDa protein could be detected at the active stage of tularemia.

On the basis of present results, the conventional LBT method was shown to be insensitive for demonstration of developing immunity. Instead, continuously growing TLL, which represent an in vitro enriched population of antigen-specific T lymphocytes, seem to be useful tools to study weak immune reactivity against a certain antigen.

The increase in the percentage of FT-Ag-induced, in vitro activated $CD4^+$ lymphocytes ($CD4^+$ DR^+) from the second to the fourth week was in harmony with the peak responsiveness of the other parameters of T-cell functions. The more marked distribution of HLA-DR antigen in $CD4^+$ T cells agrees with the known importance of $CD4^+$ cells for

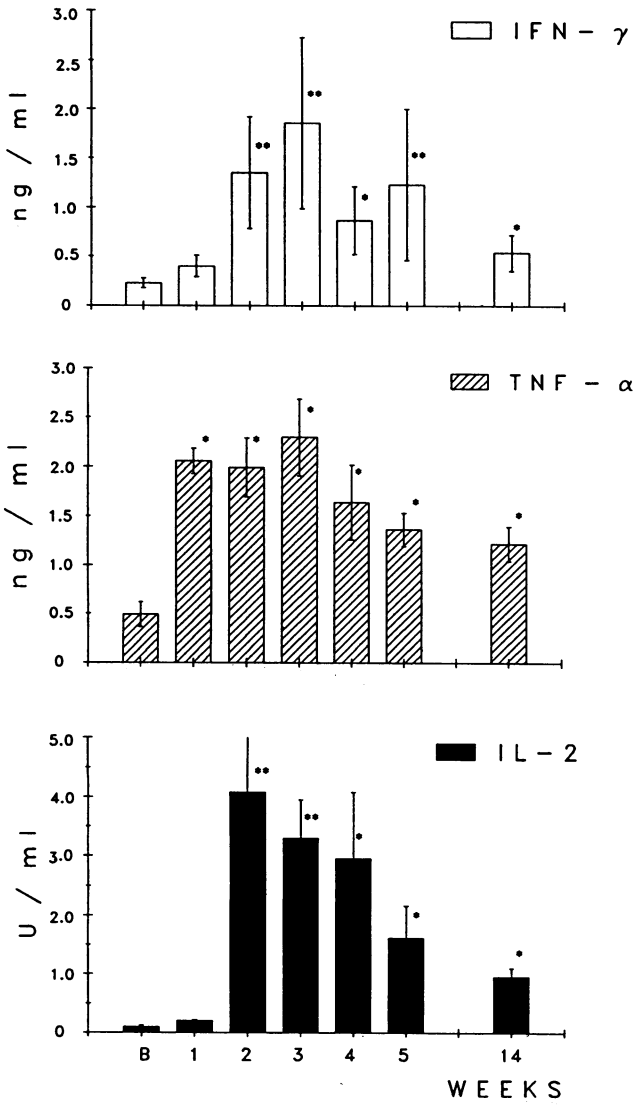

FIG. 3. Mean levels of TNF-α, IL-2, and IFN-γ production induced by FT-Ag (1 μg of protein per ml) from PBMC obtained in weeks 1 to 5 and 14 after the first tularemia symptoms (thin bars show standard errors). Significant difference from the background (B) levels is shown with asterisks: $*P < 0.01$; $**P < 0.001$.

tularemia immunity (20), which is also reflected in the $CD4^+$ phenotype of the FT-Ag-specific T-cell clones (16).

Soluble CD8, which is reported to be associated with $CD8^+$ lymphocyte activation (4), was observed to increase between 2 and 3 weeks in the FT-Ag-stimulated PBMC supernatants, but correlation with $CD8^+$ DR^+ lymphocytes could not be shown in this study.

Cytokines are important mediators in different intracellular bacterial infections. Specific T-cell reactivity against *F. tularensis* can be characterized by measuring IFN-γ or IL-2 production by FT-Ag-stimulated human T cells of established immunity (5), and we have demonstrated previously that these lymphokines are produced by T-cell clones having a CD4 phenotype (16). In the present experiments, the association of FT-Ag-induced IL-2 and IFN-γ production with the development of FT-Ag-specific T-cell proliferation was confirmed in a manner similar to that shown recently following *F. tularensis* vaccination (6).

The heterogenic nature of $CD4^+$ T-helper cells in mice has been shown to reflect in the secretion of different cytokines by Th1 and Th2 cells (10). IL-4 is produced only by Th2 cells, while IL-2 and IFN-γ are produced by Th1 cells. Our failure to find detectable IL-4 levels may indicate that Th1-like activation is more important for immune resistance against *F. tularensis*. Alternatively, it may be explained quantitatively on the basis of a recent report in which only 5% of $CD4^+$ lymphocytes expressed IL-4 mRNA, while 60% of them expressed IL-2 and IFN-γ mRNA (2).

The present results demonstrated that TNF-α was produced by FT-Ag-stimulated PBMC early in tularemia before any IFN-γ was measurable. TNF-α responses in cases of natural infection seemed to differ from those induced by a live attenuated *F. tularensis* vaccine strain, when TNF-α secretion was found after the appearances of positive FT-Ag LBT and IFN-γ responses (6). We consider the difference quantitative, caused by the ability of the wild-type *F. tularensis* strain to survive longer in phagocytes (8). The early TNF-α production in tularemia may be due to secretion of activated monocytes after phagocytosis of *F. tularensis*. This is in accordance with a recent study by Valone and coworkers (25) in which *M. bovis* BCG directly stimulated human blood monocytes to produce TNF-α very early after an antigen challenge and IFN-γ secretion followed later.

In conclusion, both the LBT and cytokine secretion responses appeared within 2 weeks after the onset of tularemia, and none of the *F. tularensis* envelope protein antigens was found to have a special role in the early phase of development of immunity during human tularemia.

ACKNOWLEDGMENT

This work was supported by the Medical Research Council, Academy of Finland.

REFERENCES

1. **Anthony, L. S. D., E. Ghadirian, F. P. Nestel, and P. A. L. Kongshavn.** 1989. The requirement for gamma interferon in resistance of mice to experimental tularemia. Microb. Pathog. **7**:421–428.
2. **Balkwill, F. R., and F. Burke.** 1989. The cytokine network. Immunol. Today **10**:299–304.
3. **Flesch, I. E. A., and S. H. E. Kaufmann.** 1988. Attempts to characterize the mechanisms involved in mycobacterial growth inhibition by gamma interferon-activated bone marrow macrophages. Infect. Immun. **56**:1464–1469.
4. **Griffin, D. E., B. J. Ward, E. Jauregui, R. T. Johnson, and A. Vaisberg.** 1989. Immune activation in measles. N. Engl. J. Med. **320**:1667–1672.
5. **Karttunen, R., G. Andersson, H.-P. T. Ekre, K. Juutinen, H.-M. Surcel, H. Syrjälä, and E. Herva.** 1987. Interleukin 2 and gamma interferon production, interleukin 2 receptor expression, and DNA synthesis induced by tularemia antigen in vitro after natural infection or vaccination. J. Clin. Microbiol. **25**:1074–1078.
6. **Karttunen, R., H.-M. Surcel, G. Andersson, H.-P. T. Ekre, and E. Herva.** 1991. *Francisella tularensis*-induced in vitro gamma interferon, tumor necrosis factor alpha, and interleukin 2 responses appear within 2 weeks of tularemia vaccination in human beings. J. Clin. Microbiol. **29**:753–756.
7. **Koskela, P., and E. Herva.** 1982. Cell-mediated and humoral immunity induced by a live *Francisella tularensis* vaccine. Infect. Immun. **36**:983–989.
8. **Löfgren, S., A. Tärnvik, G. Bloom, and W. Sjöberg.** 1983. Phagocytosis and killing of *Francisella tularensis* by human polymorphonuclear leukocytes. Infect. Immun. **39**:715–720.
9. **Magee, D., and E. J. Wing.** 1988. Cloned L3T4+ lymphocytes protect mice against Listeria monocytogenes by secreting IFN-γ. J. Immunol. **141**:3203–3207.

10. **Mosmann, T. R., and R. L. Coffman.** 1989. Heterogeneity of cytokine secretion patterns and functions of helper T cells. Adv. Immunol. **46**:111–147.
11. **Nakane, A., T. Minagawa, and K. Kato.** 1988. Endogenous tumor necrosis factor (cachectin) is essential to host resistance against *Listeria monocytogenes* infection. Infect. Immun. **56**: 2563–2569.
12. **Nash, T. W., D. M. Libby, and M. A. Horwitz.** 1988. IFN-γ activated human alveolar macrophages inhibit the intracellular multiplication of *Legionella pneumophila*. J. Immunol. **40**:3978–3981.
13. **Sandström, G., A. Tärnvik, and H. Wolf-Watz.** 1987. Immunospecific T-lymphocyte stimulation by membrane proteins from *Francisella tularensis*. J. Clin. Microbiol. **25**:641–644.
14. **Sjöstedt, A., G. Sandström, and A. Tärnvik.** 1990. Several membrane polypeptides of the live vaccine strain *Francisella tularensis* LVS stimulate T cells from naturally infected individuals. J. Clin. Microbiol. **28**:43–48.
15. **Surcel, H.-M.** 1990. Diversity of *Francisella tularensis* antigens recognized by human T lymphocytes. Infect. Immun. **58**:2664–2668.
16. **Surcel, H.-M., J. Ilonen, K. Poikonen, and E. Herva.** 1989. *Francisella tularensis*-specific T-cell clones are human leukocyte antigen class II restricted, secrete interleukin-2 and gamma interferon, and induce immunoglobulin production. Infect. Immun. **57**:2906–2908.
17. **Surcel, H.-M., M. Sarvas, I. M. Helander, and E. Herva.** 1989. Membrane proteins of *Francisella tularensis* LVS differ in ability to induce proliferation of lymphocyte from tularemia-vaccinated individuals. Microb. Pathog. **7**:411–419.
18. **Syrjälä, H., E. Herva, J. Ilonen, K. Saukkonen, and A. Salminen.** 1984. A whole-blood lymphocyte stimulation test for the diagnosis of human tularemia. J. Infect. Dis. **84**:912–916.
19. **Syrjälä, H., P. Koskela, T. Ripatti, A. Salminen, and E. Herva.** 1986. Agglutination and ELISA methods in the diagnosis of tularemia in different clinical forms and severities of the disease. J. Infect. Dis. **153**:142–145.
20. **Tärnvik, A.** 1989. Nature of protective immunity to *Francisella tularensis*. Rev. Infect. Dis. **11**:440–451.
21. **Tärnvik, A., and S. E. Löfgren.** 1975. Stimulation of human lymphocytes by a vaccine strain of *Francisella tularensis*. Infect. Immun. **20**:698–704.
22. **Tärnvik, A., G. Sandström, and S. Löfgren.** 1979. Time of lymphocyte response after onset of tularemia and after tularemia vaccination. J. Clin. Microbiol. **10**:854–860.
23. **Tracey, K. J., H. Vlassara, and A. Cerami.** 1989. Peptide regulatory factors. Cachectin/tumour necrosis factor. Lancet **i**:1122–1126.
24. **Troye-Blomberg, M., E. M. Riley, L. Kabilan, M. Holmberg, H. Perlmann, U. Andersson, C. H. Heusser, and P. Perlmann.** 1990. Production by activated human T cells of interleukin 4 but not interferon-γ is associated with elevated levels of serum antibodies to activating malaria antigens. Proc. Natl. Acad. Sci. USA **87**:5484–5488.
25. **Valone, S. E., E. A. Rich, R. S. Wallis, and J. J. Ellner.** 1988. Expression of tumor necrosis factor in vitro by human mononuclear phagocytes stimulated with whole *Mycobacterium bovis* BCG and mycobacterial antigens. Infect. Immun. **56**:3313–3315.