Low Levels of NF-κB/p65 Mark Anergic CD4⁺ T Cells and Correlate with Disease Severity in Sarcoidosis

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T lymphocytes from patients with sarcoidosis respond weakly when stimulated with mitogen or antigen. However, the mechanisms responsible for this anergy are not fully understood. Here, we investigated the protein levels of nuclear transcription factor NF-κB (p50, p65, and p105), IκBα (inhibitor of NF-κB), T-cell receptor (TCR) CD3ε-chain, tyrosine kinase p56LCK, and nuclear factor of activated T cells c2 (NF-ATc2) in peripheral blood CD4⁺ T cells from patients with sarcoidosis. Baseline expression of p65 in these lymphocytes was reduced in 50% of patients. The reduced levels of p65 in sarcoid CD4⁺ T cells concurred with decreased levels of p50, p105, CD3ε, p56LCK, IκBα, and NF-ATc2. Polyclonal stimulation of NF-κB-deficient sarcoid T cells resulted in reduced expression of CD69 and CD154, decreased proliferation, and cytokine (i.e., interleukin 2 [IL-2] and gamma interferon [IFN-γ]) production. The clinical significance of these findings is suggested by the association between low p65 levels and the development of more severe and active sarcoidosis. Although correlative, our results support a model in which multiple intrinsic signaling defects contribute to peripheral T-cell anergy and the persistence of chronic inflammation in sarcoidosis.
IκB kinase (IKK) becomes activated and phosphorylates the IκB molecules, leading to their degradation through the ubiquitin-proteosome pathway. NF-κB dimers then translocate to the nucleus and activate their target genes (22, 69).

In autoimmune diseases, chronic infections, and cancer, pathological conditions in which persistent antigen stimulation of T cells occurs, decreased expression of NF-κB, CD3ζ, and p56<sup>LCK</sup> in T lymphocytes has been implicated in the T-cell anergy associated with these diseases (9, 20, 47, 48, 71, 75). Ligation of CD152 (CTLA-4) and CD279 (PD-1), two coinhibitory molecules of the CD28 family which are expressed at increased levels on chronically stimulated T cells, can also result in functional exhaustion of T lymphocytes (12, 19). Clonally exhausted T cells were first identified in mice infected with lymphocytic choriomeningitis virus, but exhausted lymphocytes have now been found in humans with chronic infections, autoimmunity, granulomatous diseases, and cancer (17, 23, 38, 56, 72, 74).

Another lymphocyte marker that has been used to differentiate T cells according to their stimulation history is CD27. CD27 is a member of the TNF-R family and is expressed on most peripheral blood T cells. Upon antigenic restimulation, surface expression of CD27 is irreversibly lost in T cells. Thus, lack of CD27 expression is a valid surrogate marker to identify chronically stimulated T lymphocytes (3, 27).

A recent theory for the pathogenesis of sarcoidosis postulates that sarcoid granulomas are caused by an effective host response to deposition of mycobacterial antigens aggregated with host proteins. It is believed that these complexes form a poorly soluble nidus for granuloma formation that drives the pathogenic Th1 response characteristic of this disease (52). We hypothesize that failure to remove the aggregates could result in chronic stimulation of T cells and eventually in clonal exhaustion or anergy. As a first step in studying this idea, we investigated the total number and frequency of peripheral CD4<sup>+</sup> T-cell populations, the expression profiles of several key proteins involved in TCR signal transduction (i.e., NF-κB, CD3ζ, p56<sup>LCK</sup>, IκBα, and nuclear factor of activated T cells c2 [NF-ATc2]), the response of T cells to stimulation through the TCR, and the expression profiles of inhibitory receptors in T cells from sarcoid patients and healthy controls. Our results suggest that the anergic state of CD4<sup>+</sup> T lymphocytes in sarcoidosis could be a consequence of impaired signal transduction via the TCR/CD3 complex. Furthermore, we propose that by impairing CD4<sup>+</sup> T-cell effector functions, these signaling defects exert key regulatory roles that may lead to granuloma susceptibility and persistence in patients with sarcoidosis.

### MATERIALS AND METHODS

**Patients.** Twenty-two adult patients who had biopsy-proven sarcoidosis confirmed by ACCESS criteria (54) were recruited for this study. All patients had chronic persistent sarcoidosis (duration of disease, >6 years) and were either on chronic immunosuppressive therapy or not on therapy at the time of peripheral blood collection. The patients were divided into two groups: normal expressers and expressers of low levels of NF-κB dimers by their peripheral blood CD4<sup>+</sup> T cells (see Fig. 2A for the results of a representative immunoblot analysis). Clinical and demographic features of the patients with sarcoidosis are summarized in Table 1. Blood samples from age- and sex-matched healthy donors, who had no history of sarcoidosis, were collected by advertising at the local university and in the hospital employee pool. All subjects participated in the studies after signing an informed consent approved by the local ethics committee.

| TABLE 1. Clinical and demographic features of sarcoid patients |
|---------------------------------------------------------------|
| % (n) | % (n) | % (n) | % (n) | % (n) | % (n) | % (n) | % (n) |
|-------|-------|-------|-------|-------|-------|-------|-------|
| NFKBP65 level of CD4<sup>+</sup> T cells | NFKBP65 level of CD4<sup>+</sup> T cells | NF-κB level of CD4<sup>+</sup> T cells | NF-κB level of CD4<sup>+</sup> T cells | NF-κB level of CD4<sup>+</sup> T cells | NF-κB level of CD4<sup>+</sup> T cells | NF-κB level of CD4<sup>+</sup> T cells | NF-κB level of CD4<sup>+</sup> T cells |
| - | - | - | - | - | - | - | - |
| Mean age (yr) | Mean age (yr) | Mean age (yr) | Mean age (yr) | Mean age (yr) | Mean age (yr) | Mean age (yr) | Mean age (yr) |
| 47.2 | 18 | 36 (4) | 91 (10) | 91 (10) | 91 (10) | 91 (10) | 91 (10) |
| 54.1 | 9.3 | 10 (2) | 10 (2) | 10 (2) | 10 (2) | 10 (2) | 10 (2) |
| patients | reduced | reduced | reduced | reduced | reduced | reduced | reduced |
| Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Reduced | Reduced | Reduced | Reduced | Reduced | Reduced | Reduced | Reduced |
| 36 (4) | 36 (4) | 36 (4) | 36 (4) | 36 (4) | 36 (4) | 36 (4) | 36 (4) |
| 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 55 (6) | 55 (6) | 55 (6) | 55 (6) | 55 (6) | 55 (6) | 55 (6) | 55 (6) |
| 73 (8) | 73 (8) | 73 (8) | 73 (8) | 73 (8) | 73 (8) | 73 (8) | 73 (8) |
| 45 (5) | 45 (5) | 45 (5) | 45 (5) | 45 (5) | 45 (5) | 45 (5) | 45 (5) |
| 64 (7) | 64 (7) | 64 (7) | 64 (7) | 64 (7) | 64 (7) | 64 (7) | 64 (7) |
| 36 (4) | 36 (4) | 36 (4) | 36 (4) | 36 (4) | 36 (4) | 36 (4) | 36 (4) |
| 9 (1) | 9 (1) | 9 (1) | 9 (1) | 9 (1) | 9 (1) | 9 (1) | 9 (1) |
| 9 (1) | 9 (1) | 9 (1) | 9 (1) | 9 (1) | 9 (1) | 9 (1) | 9 (1) |
| 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| including lung, upper respiratory tract, skin, bone, liver, and eye. | including lung, upper respiratory tract, skin, bone, liver, and eye. | including lung, upper respiratory tract, skin, bone, liver, and eye. | including lung, upper respiratory tract, skin, bone, liver, and eye. | including lung, upper respiratory tract, skin, bone, liver, and eye. | including lung, upper respiratory tract, skin, bone, liver, and eye. | including lung, upper respiratory tract, skin, bone, liver, and eye. | including lung, upper respiratory tract, skin, bone, liver, and eye. | including lung, upper respiratory tract, skin, bone, liver, and eye. |
**Lymphocyte isolation.** Peripheral blood mononuclear cells (PBMC) were isolated from peripheral venous blood by Ficoll-Histopaque (Sigma, St. Louis, MO) density gradient centrifugation (6). Total, naïve, and memory CD4+ T cells were purified from PBMC as untouched lymphocytes using CD4+ T-cell isolation kit II and naïve and memory CD4+ T-cell isolation kits, respectively (Miltenyi Biotec). The purified lymphocyte fractions routinely contained ≥95% of the total, naïve, or memory CD4+ T cells. The CD25-depleted fractions did not contain detectable amounts of CD25+ cells as measured by flow cytometry (FC).

**Flow cytometry.** PBMC or purified CD4+ T cells (1 × 10^6) were stained for CD3e, CD4, CD19, CD25, CD28, CD45RA, CD62L, CD69, CD134, CD152, CD273, CD274, CD279 (BD Biosciences, San Diego, CA), CD27, CD45RO, CD86, CD95, CD154, or HLA-DR (eBioscience, San Diego, CA) using marker-specific fluorescent antibodies (Abs). After incubation on ice for 10 min, cells were washed twice with buffer (phosphate-buffered saline [PBS] containing 1% bovine serum albumin [BSA]), 0.01% NaN3, and resuspended in 0.5 ml of the same buffer for FC analysis. Sample acquisition was performed using a FACScan (Becton Dickinson, San Jose, CA) flow cytometer, and data were analyzed with CellQuest software (Becton Dickinson). An Fc receptor-blocking reagent (human immunoglobulins) was used to reduce nonspecific staining as indicated by the manufacturer (Miltenyi Biotec). Isotype-matched fluorochrome-labeled Abs and control Abs that were stained were used as controls. A dual-platform FC method was used to generate absolute cell counts as previously described (45).

**Immunoblotting.** Purified fractions of peripheral blood CD4+ T cells (total, naïve, or memory), CD4+ T-cell-depleted PBMC fractions, or Jurkat and K562 cells were extracted in 1% Triton X-100 lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 10% glycerol, 1 mM sodium orthovanadate, 10 mM β-glycerophosphate, and a protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). Protein extracts were obtained by centrifugation at 15,000 rpm for 15 min at 4°C and stored at −80°C. Protein concentration was determined by standard colorimetric assay (Bio-Rad Laboratories, Richmond, CA). Twenty micrograms of purified total protein was loaded and separated by 10 to 12% SDS-PAGE. Separated proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane and immunodetected with monoclonal Abs to PP2A (Millipore, Billerica, MA), the p50/p65 subunits of NFκB/p50/p105 (C-terminal epitope), and NFκB/p65 (N-terminal epitope) (Cell Signaling Technology, Danvers, MA), NFκBp65 (C-terminal epitope), actin, CD3, NFκB, or p56+ (Santa Cruz Biotechnology, Santa Cruz, CA), according to the manufacturers’ recommendations. Films were scanned and the densities of the bands measured with LabWorks 4.6 software (UVP LLC, Upland, CA).

**Lymphocyte activation and proliferation assays.** Fractions of peripheral blood CD4+ T cells that were either depleted or not depleted of CD25+ cells were labeled with 10 μM carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen, Carlsbad, CA), or a previously described Tetramer (data not shown). Cells were suspended in RPMI 1640 medium (GIBCO BRL, Grand Island, NY), containing 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and 1 mM sodium pyruvate with antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) and then plated in 24-well culture plates (0.5 cell l) for 1 to 4 days, were harvested and CD4+ cell number was measured using FlowMoc (In vitroDynal, Milpitas, CA). Cells, incubated at 37°C in humidified air plus 5% CO2 for 1 to 4 days, were harvested and CD3/28 beads removed using DynaMag (Invitrogen/Dynal). Subsequently, cells were stained for CD25, CD69, CD86, CD95, CD134, CD152, CD273, CD274, CD279, or HLA-DR, as described above, and analyzed by FC. In experiments in which anti-CD152 or anti-CD274 (eBioscience) was used to block inhibitory receptors, 1 μg/ml of Ab was added to the cultures. CD4+ T cells were also treated with anti-CD152 or anti-CD274 to monitor for the cytotoxic effects of each of these reagents, of which none were noted.

**Real-time PCR arrays.** Total RNA was extracted from activated CD4+ T cells using TRIzol reagent (Invitrogen). For the real-time PCR (RT-PCR), an aliquot of 1 μg of total RNA was reverse transcribed using an RT2 First Strand kit (SA Biosciences, Frederick, MD). cDNA from all samples were mixed with RT2 quantitative PCR (qPCR) master mix (SA Biosciences), according to the manufacturer’s instructions. The PCR was performed on an Applied Biosystems 7300 RT-PCR system (Applied Biosystems, Foster City, CA) using the following conditions: 1 cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, with a final cycle at 60°C for 1 min. RNA specimens were analyzed in duplicate using primer sets for human IL-2 (SA Biosciences catalog no. PHH00712B; IL-4 (PHH00565A), gamma interferon (IFN-γ) (PHH00380B), and Foxp3 (PHH00029B). Actin (PHH00073E) expression was used as a reference to normalize target gene expression levels. Data were analyzed using SuperArray BioScience software (SA Biosciences). Results are presented as fold changes in mRNA expression relative to control values.

**Statistical analysis.** Statistical comparisons were performed using the non-parametric Mann-Whitney U test (GraphPad Prism 5.0; GraphPad Software, Inc., La Jolla, CA), and significance was defined as a P < 0.05.

### RESULTS

**Increased number and frequency of blood memory CD4+ T cells in sarcoidosis.** Surface staining of CD4+ T cells with monoclonal Abs to CD62L and CD45RA or CD45RO allows the identification of three distinct T-cell subpopulations characterized by different homing, proliferative, and effector functions: naïve T cells (CD62L+CD45RA−CD45RO+), central memory T cells (TCM) (CD62L+CD45RA−CD45RO+), and effector memory T cells (TEM) (CD62L−CD45RA−CD45RO−) (18, 60). To investigate whether the frequency and absolute number of these T-cell subsets were altered in sarcoidosis patients, we determined and compared the percentages and total numbers of naïve, TEM, and TEM lymphocytes in the peripheral blood of sarcoid patients and healthy controls using dual-platform FC. A representative example of the immunofluorescent staining for CD4, CD45RA, and CD62L is shown in Fig. 1A. Compared with healthy controls, sarcoid patients had a significantly reduced frequency (32.7 ± 6.1 versus 24.3 ± 10.7) (Fig. 1B) but normal absolute numbers (1,623 ± 654.8 versus 1,325 ± 1,389 cells/μl; P = 0.0856) of CD4+ T cells (data not shown). Both the frequency (56.3 ± 12.2 versus 35.1 ± 15.6) and the absolute number (1,005 ± 482.6 versus 436.5 ± 449.3 cells/μl) of naïve CD4+ T cells were significantly decreased in patients with sarcoidosis (Fig. 1C and D). In contrast, the frequency (8.7 ± 2.8 versus 12.8 ± 6.7) and total number (57.1 ± 63.4 versus 207.1 ± 206.9 cells/μl) of TEM cells were significantly increased in sarcoid patients (Fig. 1E and F). Compared with healthy controls, patients with sarcoidosis exhibited a significantly increased frequency (33.2 ± 10.3 versus 47 ± 12.5) of TEM lymphocytes (Fig. 1G). The absolute number (205.1 ± 139.4 versus 400.5 ± 487.6 cells/μl) of TEM cells was also increased in sarcoid patients, but this difference did not reach statistical significance (data not shown). A comparative analysis of CD27 expression in PBMC from sarcoid patients and healthy controls revealed a significantly increased frequency (5.1 ± 3.9 versus 13.6 ± 9.2) of CD27+ lymphocytes within the memory CD4+ cell subset of patients with sarcoidosis (Fig. 1H). Additionally, sarcoid patients exhibited a significantly increased proportion (0.7 ± 1.2 versus 7.6 ± 9.8; P = 0.0036) of CD4+ T cells expressing the CD27−CD45RO+ phenotype. The CD27−CD45RO+ cells were almost absent from the peripheral CD4+ population of healthy controls (<1%). A representative example of the immunofluorescent staining for CD4, CD45RO, and CD27 is shown in Fig. 1I. The accumulation of memory CD4+ T cells lacking CD27 expression suggest accelerated differentiation of naïve T cells into memory cells and persistent antigenic stimulation in sarcoidosis.

**Peripheral blood CD4+ T cells from patients with sarcoidosis exhibit decreased levels of NR-KB/p65.** To investigate whether signaling defects associated with persistent antigenic stimulation were responsible for the peripheral T-cell anergy observed in sarcoidosis patients, immunoblotting of whole-protein lysates of peripheral blood CD4+ T cells isolated from...
sarcoid patients or controls was performed using a monoclonal Ab specific to NF-κB/p65 (Fig. 2A). We found that p65 protein levels were significantly reduced or absent in 50% of the sarcoid patients analyzed. The intensity of the p65 band in the remaining 50% of sarcoid patients was quantitatively comparable to that of normal controls. Using protein extracts from the same patients and controls, we reassessed the levels of p65 with another anti-p65 monoclonal Ab that recognizes the N terminus of the protein (Fig. 2B). We again noted the reduction or absence of the p65 protein in these peripheral blood sarcoid CD4 T cells, indicating that defective p65 expression was not due to a loss or modification of a specific epitope. In contrast, p65 protein was detected, albeit at somewhat reduced levels, in lysates of CD4-depleted PBMC from these sarcoid patients, suggesting that the defect was predominant, but not limited, to peripheral blood CD4 T cells (Fig. 2C). Next, we determined the amounts of NF-κB (p105 and p50), p56LCK, NF-ATc2, PP2A, and CD3ζ in peripheral blood CD4 T cells from sarcoid patients and controls by immunoblotting. As shown in Fig. 2A, D, and E, protein expression levels of p105, p50, p56LCK, NF-ATc2, and CD3ζ were reduced predominantly in the group of sarcoid patients that exhibited decreased levels of p65. The reduced levels of p105, p50, p56LCK, NF-ATc2, and CD3ζ were not the result of a global defect in the expression of signaling molecules in sarcoid T cells, since levels of the protein phosphatase PP2A were comparable in all peripheral blood CD4 T cells analyzed (Fig. 2D). In addition, immunoblot analysis of lysates of naïve and memory CD4 T cells isolated from sarcoid patients and controls revealed that the p65 deficiency was not restricted to the memory population since naïve cells exhibited the exact same defect (Fig. 2F). In one patient, however, lack of p65 was limited to naïve cells, but longer exposure of the same blot revealed the presence of a faint p65 band (data not shown). Finally, association studies demonstrated that p65-deficient sarcoid patients had higher (57.1 ± 63.4 versus 150.5 ± 148.9 cells/μl) absolute numbers of...
TEM cells than healthy controls, but this difference was not statistically significant (Fig. 2G). Thus, a considerable proportion of sarcoid patients showed an abnormal expression of p65 in naïve and memory CD4 T cells, and the p65 defect was associated with a specific decrease in the levels of various signaling molecules (i.e., p105, p50, p56 LCK, NF-ATc2, and CD3).</p><p>Impaired responsiveness of NF-κB/p65-deficient sarcoid CD4 T cells to TCR stimulation. In T lymphocytes, NF-κB function is critical for the control of a variety of cellular processes, including activation, proliferation, and cytokine production (22, 66, 69). It was hypothesized, therefore, that decreased levels of p65 in peripheral blood sarcoid T lymphocytes would render these cells functionally impaired. To investigate this, the effect of p65 deficiency on CD4 T-cell activation, proliferation, and cytokine production was examined. Purified preparations of p65-deficient or p65-competent sarcoid CD4 T cells and CD4 T cells from healthy controls were stimulated for 24 h with CD3ε/CD28 beads, and the cells were then analyzed by FC for levels of expression of CD25, CD69, CD134, CD154, and HLA-DR. Each of these surface markers is known to be upregulated by CD4 T cells upon antigenic stimulation (35, 59, 68). In comparison to p65-competent CD4 T cells, stimulation of p65-deficient sarcoid CD4 T cells with CD3ε/CD28 beads resulted in significantly reduced expression of CD69 and CD154 (Fig. 3A and B). In contrast, stimulation with CD3ε/CD28 beads significantly increased the expression of HLA-DR in p65-deficient sarcoid CD4 T cells (Fig. 3C). After CD3ε/CD28 stimulation, no significant difference in the levels of expression of CD25 and CD134 were observed between p65-deficient sarcoid CD4 T cells and p65-competent CD4 T cells from sarcoid patients and healthy controls (see Fig. S1A and B in the supplemental material). After 4 days of culture, the CD4 T cells were
analyzed by FC for proliferation by use of CFSE staining. Cells which have divided exhibit less CFSE fluorescence due to intracellular dilution of the stain (43). Stimulation of p65-deficient sarcoid CD4^+ T cells with CD3ε/CD28 beads was associated with substantially less cell proliferation than what was observed for p65-competent CD4^+ T cells (Fig. 3D). The reduced proliferation of p65-deficient sarcoid CD4^+ T cells was not due to a higher fraction of T_{EM} cells, which are incapable of vigorous proliferation; we did not observe any significant difference between the frequencies of T_{EM} lymphocytes within the populations of p65-deficient and p65-competent CD4^+ T cells (Fig. 3E). Moreover, cultures of p65-deficient CD4^+ T cells that were depleted of Treg cells exhibited levels of proliferation equivalent to those seen in nondepleted cultures, suggesting that the reduced lymphoproliferation observed in cultures of p65-deficient sarcoid CD4^+ T cells was not the result of the suppressive actions of Treg cells that possibly contaminated these cultures. In accordance with this effect, levels of IL-2 and IFN-γ mRNA were significantly reduced in p65-deficient sarcoid CD4^+ T cells in comparison to levels of IL-2 and IFN-γ mRNA found in CD4^+ T cells from healthy controls (see Fig. S2A and B in the supplemental material). To further investigate the effect of p65 deficiency on the function of sarcoid T cells, cultures of p65-deficient sarcoid CD4^+ T cells were stimulated with CD3ε/CD28 beads for 6 h. In comparison to levels of IL-2 and IFN-γ mRNA in p65-competent CD4^+ T cells, levels of IL-2 and IFN-γ mRNA were significantly reduced in p65-deficient sarcoid CD4^+ T cells.

FIG. 3. Impaired activation, proliferation, and cytokine production of NF-κB/p65-deficient sarcoid T cells. CD4^+ T lymphocytes, purified from PBMC of sarcoid patients and healthy controls by MACS, were incubated for 24 h with CD3ε/CD28 beads, and the cells were then analyzed by FC for levels of expression of CD69 (A), CD154 (B), and HLA-DR (C). (D) Blood CD4^+ T cells, purified either from sarcoid patients or from healthy controls, were labeled with CFSE and stimulated with CD3ε/CD28 beads for 4 days, and the mean fluorescence intensity (MFI) of CFSE staining was determined by FC in the CD4^+ T-cell population. (E) Relationship between p65 status and frequency of T_{EM} cells in sarcoid patients and controls. Levels of IL-2 (F) and IFN-γ (G) mRNA were determined by real-time PCR in purified CD4^+ T cells isolated from sarcoid patients and controls after stimulation of these cells with CD3ε/CD28 beads for 6 h. (H) Levels of CD95 were determined by FC in the separated CD4^+ T cells after stimulation of these lymphocytes with CD3ε/CD28 beads for 24 h. The median MFI values for CD69, CD154, HLA-DR, CD95, and CFSE staining are shown as solid lines. Representative histograms are shown. (A to D, bottom) Thin line, isotype control (A, B, and C) or CFSE-labeled unstimulated cells (D); thick line, healthy control cells; black, NF-κB^+ cells; gray, NF-κB^- cells.
cells (Fig. 3F and G). In contrast, levels of IL-4 mRNA in p65-deficient sarcoid CD4⁺ T cells were higher than in p65-competent CD4⁺ T cells, although the difference was not statistically significant (data not shown). The impaired responsiveness of p65-deficient sarcoid CD4⁺ T cells could not be attributed to diminished expression of the coreceptors CD3ε and CD28 on these cells; p65-deficient and p65-competent CD4⁺ T cells expressed equivalent levels of these two molecules (see Fig. S3 in the supplemental material). After stimulation with CD3ε/CD28 beads, significantly increased levels of CD95 were observed in p65-deficient sarcoid CD4⁺ T cells in comparison to those seen in p65-competent CD4⁺ T cells (Fig. 3H). No significant differences in cell viability, as determined by trypan blue exclusion assay, were found between cultures of p65-deficient and p65-competent CD4⁺ T cells (data not shown). Compared with levels in control CD4⁺ T cells (Fig. 4A), when sarcoid CD4⁺ T cells were stimulated with CD3ε/CD28 beads, however, significantly increased levels of CD152 were detected in these cells in comparison to levels in identically stimulated CD4⁺ T cells from healthy controls (Fig. 4B). In comparison with no stimulation, levels of CD279 in stimulated sarcoid CD4⁺ T cells were significantly higher than those expressed by unstimulated p65-competent CD4⁺ T cells (Fig. 4B). In contrast, levels of CD86 in stimulated sarcoid CD4⁺ T cells were significantly lower than those observed in stimulated CD4⁺ T cells from healthy controls (Fig. 4A). When sarcoid CD4⁺ T cells were stimulated with CD3ε/CD28 beads, however, significantly increased levels of CD152 were detected in these cells compared to levels in identically stimulated CD4⁺ T cells from healthy controls (Fig. 4B). In sarcoid CD4⁺ T cells that were either stimulated or not with CD3ε/CD28 beads, levels of CD273 and CD274 (two CD279 ligands) were low (CD274) or negative (CD273) and did not significantly differ from those observed in control CD4⁺ T cells (data not shown). Compared with levels in control CD4⁺ T lymphocytes, significantly increased levels of CD86 (a ligand for CD152) were found in both resting and activated p65-deficient sarcoid CD4⁺ T cells (Fig. 4C). Since stimulation with CD3ε/CD28 beads significantly enhanced the levels of CD86 and CD152 expressed on p65-deficient sarcoid CD4⁺ T cells, it was possible that increased levels of these molecules could facilitate direct interactions between T cells through CD86-CD152 interactions (see Fig. S5A and B in the supplemental material). Taken together, these data suggest that p65-deficient sarcoid CD4⁺ T cells are exhausted lymphocytes but...
that the reduced proliferation exhibited by these cells was not the result of negative signals delivered by the coinhibitory receptor CD152.

**NF-κB/p65 levels and clinical variables of disease activity.** Immunoblot analysis revealed that half of the sarcoid patients exhibited deficient p65 expression in their peripheral blood CD4⁺ T cells. To examine the clinical relevance of this laboratory finding, we investigated possible associations between p65 protein levels and clinical variables of disease activity in sarcoidosis (Table 1). Compared to what we observed in p65-competent patients, multiple (sarcoidosis) (Table 1). Compared to what we observed in p65-competent patients, multiple (sarcoidosis) compared to p65-competent patients or healthy controls (Fig. 5A). Compared to p65-competent patients, p65-deficient sarcoid CD4⁺ T cells were significantly lower than those found in p65-competent CD4⁺ T cells and that levels of IκBα protein in sarcoid CD4⁺ T lymphocytes did not correlate with steroid treatment (Fig. 6D). We did not find any associations between the use of prednisone and increased frequency (data not shown) and numbers of naïve CD4⁺ T cells (Fig. 6I). Furthermore, no associations were found between the use of hydroxychloroquine or methotrexate and reduced levels of NF-κB (p50, p65, and p105) and CD3ζ, or between the use of hydroxychloroquine or methotrexate and increased frequency (data not shown) and numbers of naïve CD4⁺ T cells (Fig. 6I). These results suggest that therapy, for the most part, cannot be held accountable for the molecular and cellular dysfunctions observed in p65-deficient sarcoid T cells.

**DISCUSSION**

Patients with sarcoidosis exhibit a depressed systemic cellular immunity manifested by impaired T-cell responses to mitogens and recall antigens (16, 34). Here, we demonstrate that this anergic state correlates with multiple intrinsic defects in peripheral blood CD4⁺ T cells. To our knowledge, these results represent the first analysis of the expression of NF-κB, IκBα, CD3ζ, p56LCK, and NF-ATc2 in highly purified preparations of sarcoid CD4⁺ T lymphocytes.

Biochemical analysis of sarcoid CD4⁺ T cells revealed reduced p65 protein levels in 50% of the patients. The reduced p65 levels concurred with deficient expression of NF-κB (p50 and p105), IκBα, CD3ζ, p56LCK, and NF-ATc2 and with decreased responses of these lymphocytes to TCR stimulation. Compared to p65-competent CD4⁺ T cells, p65-deficient sarcoid CD4⁺ T cells proliferated less and expressed lower levels of IL-2 and IFN-γ mRNA following activation. The presence of κB binding sites in the promoters of IL-2 and IFN-γ suggest that p65 deficiency in sarcoid T cells could result in reduced expression of these cytokine genes upon stimulation (25, 63). Whether reduced levels of p65, p56LCK, NF-ATc2, or CD3ζ in sarcoid T cells were directly responsible for the anergy of these lymphocytes is unclear. However, the functional defects observed in transgenic mice in which p65/p50 complexes were selectively inhibited in T cells demonstrates that NF-κB deficiency may induce an anergic state in T cells (1, 14). Restoration experiments are under way in our laboratory to demon-
strate a cause-effect relationship between reduced levels of p65, p50, NF-ATc2, and CD3 and T-cell anergy in sarcoidosis patients.

The magnitude of T-cell division is partially determined by the relative proportions of memory T-cell subsets in the blood. In chronic beryllium disease and AIDS, a reduced lymphoproliferation correlated with an increased frequency of TEM cells (21, 73). However, reduced division of p65-deficient sarcoid T cells was not the result of an increased proportion of TEM cells, which are incapable of vigorous proliferation; the frequencies of blood TEM cells were similar in all sarcoid patients analyzed regardless of levels of p65 protein in their CD4+ T lymphocytes. Because Treg cells accumulate in the peripheral blood of patients with sarcoidosis and chronic exposure to antigen upregulates the expression of inhibitory receptors in T cells (17, 36, 50, 56), it is possible that Treg-cell-mediated suppression or reception of negative signals through inhibitory receptors could have induced T-cell anergy in sarcoidosis patients. None of these mechanisms were responsible for the observed anergy, since depletion of Treg cells and blockage of CD152-CD80/CD86 or CD279-CD274 interactions did not restore the proliferation of p65-deficient sarcoid T lymphocytes. Since NF-AT is a critical factor involved in the protection of T cells from apoptosis, p65 deficiency could have caused increased apoptosis in stimulated T lymphocytes (5, 69). We, however, did not observe differences in levels of cell viability between cultures of p65-deficient and p65-competent T cells, ruling out the possibility that an increased apoptotic rate is responsible for the reduced lymphoproliferation of p65-deficient sarcoid T cells. Since upregulation of CD152 on activated T cells can induce resistance to apoptosis (57) and p65-deficient sarcoid T cells exhibited increased levels of...
CD152 expression, it is possible that upregulation of CD152 in p65-deficient sarcoid T cells might have protected these lymphocytes from apoptosis.

Corticosteroids suppress NF-κB activity by increasing the level of IκBα (2, 61). However, the possibility of a role for steroids in the observed decrease in p65 levels can be excluded on the basis of the following observations. First, steroids have no effect on the total amount of cellular p65 protein (61). Second, the use of whole-protein lysates and reducing conditions in the immunoblot assay ruled out sequestration of p65 in the cytoplasm by the inhibitor IκBα. Third, p65-deficient sarcoid T cells expressed significantly reduced levels of IκBα protein, and levels of IκBα protein did not correlate with steroid treatment. Fourth, low p65 levels were found in some patients who were off steroids. Fifth, some patients who were taking prednisone displayed normal p65 levels. Interestingly, sarcoid patients who exhibited reduced p65 levels required more immunosuppression (i.e., prednisone, hydroxychloroquine, and methotrexate) than those with normal levels of that same protein, perhaps as a result of increased disease activity or severity (i.e., radiographic evidence of pulmonary fibrosis [stage IV pulmonary sarcoidosis], worsening disease, need for combination therapy, and greater extrapulmonary involvement). This may explain the association between low p65 protein levels and steroid therapy found in this study. Since steroids inhibit NF-κB signaling and since our p65-deficient sarcoid CD4⁺ T cells already had low IκBα and NF-κB protein levels, it is possible that these lymphocytes are insensitive to the effects of steroids because they use alternative pathways to activate their effector functions. Consequently, low p65 protein levels in blood CD4⁺ T cells could possibly be used as a marker to identify a subset of sarcoid patients who will probably not benefit from steroid therapy. Hydroxychloroquine and methotrexate, on the other hand, suppress T-cell responses by inhibiting antigen presentation and cell proliferation, respectively, but these two disease-modifying antirheumatic drugs have no effects on the basal level of p65 protein in T lymphocytes (51). Interestingly, a recent report indicated that absolute lymphopenia in sarcoid patients correlates with severe disease manifestations and was not related to medical therapy (65). In accordance with these results, we found that sarcoid patients who exhibited low p65 levels also had absolute lymphopenia that was not associated with any specific medical treatment. Our results suggest that low p65 protein levels and reduced CD4⁺ and CD19⁺ lymphocyte counts could be used as novel surrogate markers for disease severity in sarcoidosis.

The CD3ζ chain plays a key role in TCR assembly, expression, and signaling (4, 13). In spite of the reduced CD3ζ protein levels observed in p65-deficient sarcoid CD4⁺ T cells, CD3ζ surface expression in these lymphocytes was unaffected. These results indicate that down-modulation of the TCR-CD3 complex was not responsible for the anergy exhibited by p65-deficient sarcoid T cells following CD3ζ ligation. Because sustained exposure of mice to bacterial antigens induces CD3ζ down-modulation and improves T-cell function (8), reduced expression of CD3ζ in sarcoid T cells could be a direct consequence of persistent antigenic stimulation of these lymphocytes in the granulomas. The predominant memory phenotype and increased frequency of differentiated (CD27⁺) effector cells observed within the peripheral blood CD4⁺ subset of patients with sarcoidosis, as well as the increased levels of CD95, CD152, and HLA-DR expressed by these lymphocytes, support this notion. However, since the frequency of antigen-specific T cells in patients with sarcoidosis may be low (11), it is unlikely that a general decrease in CD3ζ expression in blood lymphocytes from patients with sarcoidosis arises only from T-cell populations which have encountered their specific antigens. In support of this idea, we found that reduced levels of p65 were not restricted to the population of memory CD4⁺ T cells but that naïve CD4⁺ T cells also exhibited the same defect. Because chronic exposure of T cells to TNF-α and hydrogen peroxide induce down-modulation of CD3ζ expression and T-cell anergy (33, 55), it is possible that production of these factors within the granulomas could have induced CD3ζ down-modulation in nonspecific T cells that have been recruited to the areas of granulomatous inflammation.

The engagement of the B-cell surface molecule CD40 by its T-cell counter receptor CD154 has a central role in the activation, expansion, and differentiation of B cells participating in T-cell-dependent responses (41, 58). Since NF-ATc2 levels were significantly reduced in p65-deficient sarcoid T cells, and because NF-ATc2 is essential for activation of CD154 gene expression (39, 62), reduced levels of NF-ATc2 could be directly responsible for the down-modulation of CD154 expression observed in these lymphocytes. If an effective B-cell response is essential for clearing pathogenic antigens in sarcoidosis, then reduced expression of CD154 in sarcoid CD4⁺ T cells may induce a defective Ab response that cannot eliminate antigens that induce granuloma formation. Similarly, engagement of CD40 on macrophages by CD154 results in the production of nitric oxide, TNF-α, and IL-12, three critical mediators involved in the elimination of intracellular antigens and the promotion of Th1-mediated inflammation (26). Thus, deficient expression of NF-ATc2 by sarcoid T cells has the potential to impair macrophage activation and elicitation of cell-mediated immune responses, resulting in antigen persistence and chronic inflammation.

It is difficult to envision how the observed dysfunction of T cells can coexist with local (i.e., lung, skin, etc.) excessive responses that occur in sarcoidosis patients. It is possible that in order to control the inflammation, the immune system works toward a state of suppression of T-cell function by down-modulating the expression of signaling molecules in T lymphocytes but fails to do so adequately (7). Consequently, a low level of T-cell function can still be detrimental. The signaling defects could induce a disturbed balance between Th1-cell subpopulations. If such balances are critical for sarcoidosis, then a preponderance of dysfunctional Th1 cells at target organs may be an important determinant of susceptibility and the persistence of inflammation in patients with sarcoidosis.

Preliminary studies in our laboratory showed that some sarcoid patients who had p65 deficiency in their peripheral blood T cells exhibited the exact same defect in their bronchoalveolar lavage T cells. These results suggest that the altered signal transduction phenotype observed in peripheral blood T cells of patients with sarcoidosis is not merely an epiphenomenon but also a characteristic of T cells found at the primary target organ (i.e., lungs). We hypothesize that these signaling defects could be responsible for a selective immune deficiency state that might predispose patients with chronic pulmonary sarcoid-
osis to an increased risk of *Asperillus* infection and development of mycetomas (70).

In summary, our study identified multiple intrinsic defects in blood CD4+ T cells from patients with sarcoidosis. The biochemical and functional status of these lymphocytes resembles the unresponsive state of T cells in patients with AIDS, cancer, and autoimmunity, and indicates features suggestive of anergy. The alteration of T-cell signaling is probably related, at least in part, to conditions of chronic antigen stimulation, perhaps by persistent sarcoid antigens, superantigens, and/or autoantigens in the granulomas. Chronic exposure of CD4+ T lymphocytes to elevated levels of TNF-α and hydrogen peroxide may also play a role in the signaling defects. The significance of the T-cell signaling defects for the pathogenesis of sarcoidosis is unclear, but we believe that reduced, rather than overactive, T-cell signaling defects for the pathogenesis of sarcoidosis is more likely. The alteration of T-cell signaling is probably related, at least in part, to the chronic exposure of CD4+ T cells to TNF down-regulation of T-cell receptor zeta chains and p56lck (46). T-cell receptor zeta chain downregulation: curtailing an excessive inflammatory immune response. Nat. Rev. Immunol. 4:675–687.

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**REFERENCES**

1. Aronica, M. A., et al. 1999. Preferential role for NF-kappa B/Rel signaling in the type I but not type II T cell-dependent immune response in vivo. J. Immunol. 163:5116–5122.

2. Auphan, N., J. A. DiDonato, C. Rosette, A. Helmberg, and M. Karin. 1995. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 270:286–290.

3. Baars, P. A., M. M. Maurice, M. Rep, B. Hooibrink, and R. A. van Lier. 1999. Regulation of CD27 expression on subsets of human T-cells infiltrating human renal cell carcinoma. Cancer Res. 59:4124–4129.

4. Baniyash, M. 1999. Preferential role for NF-kappaB/Rel signaling in the type 1 but not type 2 T cell-dependent immune response in vivo. J. Immunol. 162:1473–1478.

5. B Postsip, N., J. Wahlstrom, A. Eklund, and J. Grunewald. 1997. T cells in rheumatoid arthritis. Br. J. Rheumatol. 36:617–619.

6. Bronstein-Siton, N., et al. 2003. Sustained exposure to bacterial antigen induces interferon-gamma-dependent T cell receptor zeta down-regulation and impaired T cell function. Nat. Immunol. 4:957–964.

7. Brundula, V., et al. 1999. Diminished levels of T cell receptor zeta chains in peripheral blood T lymphocytes from patients with systemic lupus erythematosus. Arthritis Rheum. 42:1008–1016.

8. Corn, R. A., et al. 2003. T-cell intrinsic requirement for NF-kappa B induction of postdifferentiation IFN-gamma production and clonal expansion in a Th1 response. J. Immunol. 171:1816–1824.

9. Cox, C. E., A. Davis-Allen, and M. A. Judson. 2005. Sarcoidosis. Med. Clin. North Am. 89:517–528.

10. Daniele, R. P., J. H. Dauber, and M. D. Rossman. 1980. Immunologic abnormalities in sarcoidosis. Ann. Intern. Med. 92:900–916.

11. Day, C. L., et al. 2006. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 443:350–354.

12. Fekete, A., et al. 2007. CD4+ T cell homeostasis in rheumatoid arthritis: suggested relationships with antigen-driven immune responses. J. Autoimmun. 29:154–163.

13. Fife, B. T., and J. A. Bluestone. 2008. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunol. Rev. 224:166–182.

14. Finke, J. H., et al. 1993. Loss of T-cell receptor zeta chain and p56lck in T cells infiltrating human renal cell carcinoma. Cancer Res. 53:5613–5616.

15. Fontenot, A. P., et al. 2005. Frequency of beryllium-specific, central memory CD4+ T cells in blood determines proliferative response. J. Clin. Invest. 115:2886–2893.

16. Gerondakis, S., R. Grumont, I. Rourke, and M. Grossmann. 1998. The regulation and roles of CD154-bacterial antigen transcription factors during lymphocyte activation. Curr. Opin. Immunol. 10:533–539.

17. Golden-Mason, L., et al. 2007. Upregulation of PD-1 expression on circulating and intrahepatic hepatitis C virus-specific CD8+ T cells associated with reversible immune dysfunction. J. Virol. 81:9249–9258.

18. Goodwin, J. S., R. DeHortusu, M. Israel, G. T. Peake, and R. P. Messner. 1979. Suppressor cell function in sarcoidosis. Ann. Intern. Med. 90:169–173.

19. Granelli-Piperno, A., and P. Nolan. 1991. Nuclear transcription factors that bind to elements of the IL-2 promoter. Induction requirements in primary human T cells. J. Immunol. 147:2734–2739.

20. Grelew, I. S., and R. A. Flavell. 1998. CD40 and CD154 in cell-mediated immunity. Annu. Rev. Immunol. 16:111–135.

21. Hintzen, R. Q., et al. 1993. Regulation of CD27 expression on subsets of murine T-lymphocytes. J. Immunol. 151:2426–2435.

22. Hudspith, B. N., J. Brostoff, M. W. McNicol, and N. M. Johnson. 1984. Anergy in sarcoidosis: the role of interleukin-1 and prostaglandins in the depressed in vitro lymphocyte response. Clin. Exp. Immunol. 57:324–330.

23. Hughes, C. C., and J. S. Poher. 1996. Transcriptional regulation of the interleukin-2 gene in normal human peripheral blood T cells. Convergence of costimulatory signals and differences from transformed T cells. J. Biol. Chem. 271:5369–5377.

24. Hunninghake, G. W., and R. G. Crystal. 1981. Mechanisms of hypergammaglobulinemia in pulmonary sarcoidosis. Site of increased antibody production and role of T lymphocytes. J. Clin. Invest. 67:86–92.

25. Hunninghake, G. W., and R. G. Crystal. 1981. Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. N. Engl. J. Med. 305:429–434.

26. Iannuzzi, M. C., B. A. Rybicki, and A. S. Teirstein. 2007. Sarcoidosis. N. Engl. J. Med. 357:2153–2165.

27. Isomaki, P., et al. 2001. Prolonged exposure of T cells to TNF down-regulates TCR zeta chain and implies a role of the TCR/CD3 complex at the cell surface. J. Immunol. 166:4595–4597.

28. Kataria, Y. P., A. L. Sagone, A. G. LoBuglio, and P. A. Bramberg. 1973. In vitro observations on sarcoid lymphocytes and their correlation with cutaneous energy and clinical severity of disease. Am. Rev. Respir. Dis. 106:767–776.

29. Katchar, K., J. Wahleström, A. Eklund, and J. Grunewald. 2001. Highly activated T-cell receptor AV228 (+) CD4 (+) lung T-cell expansions in pulmonary sarcoidosis. Am. J. Respir. Crit. Care Med. 163:1540–1545.

30. Kauffman, D. E., et al. 2007. Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. Nat. Immunol. 8:1246–1254.

31. Kizian, A., et al. 2004. Expression and regulation of NFAT (nuclear factors of activated T cells) in human CD34+ cells: down-regulation upon myoid differentiation. J. Leukoc. Biol. 76:1057–1065.

32. Kobayashi, M., et al. 2005. Enhanced expression of programmed death-1 (PD-1)/PD-L1 in salivary glands of patients with Sjogren's syndrome. J. Rheumatol. 32:2156–2163.

33. Kyttila, V. C., Y. Wang, Y. T. Juang, A. Weinstein, and G. C. Tsooks. 2007. Increased levels of NF-ATc2 differentially regulate CD154 and IL-2 genes in T cells from patients with systemic lupus erythematosus. J. Immunol. 178:1960–1966.

34. Lai, J. H., et al. 1995. RelA is a potent transcriptional activator of the interleukin 2 promoter. Mol. Cell. Biol. 15:4171–4177.

35. Longarci, V., R. Badolo, S. Ferrari, and A. Plebani. 2005. Hyper immunoglobulin M syndrome due to CD40 deficiency: clinical, molecular, and immunological features. Immunol. Rev. 203:48–66.
42. Lu, L. F., and A. Rudensky. 2009. Molecular orchestration of differentiation and function of regulatory T cells. Genes Dev. 23:1270–1282.
43. Lyons, A. B., and C. R. Parish. 1994. Determination of lymphocyte division by flow cytometry. J. Immunol. Methods 171:131–137.
44. Maggi, S. B., E. W. Harbaj, and S. C. Sun. 1997. Regulation of the interleukin-2 CD28-responsive element by NF-ATp and various NF-kappaB/Rel transcription factors. Mol. Cell. Biol. 17:2605–2614.
45. Mandy, F., G. Janosy, M. Bergeron, R. Pilon, and S. Fauchier. 2008. Affordable CD4 T-cell enumeration for resource-limited regions: a status report for 2008. Cytometry B Clin. Cytom. 74(Suppl. 1):S27–S39.
46. Mathew, S. K., L. Bauer, A. Fischheder, N. Bhardwaj, and S. J. Oliver. 2008. The anergic state in sarcoidosis is associated with diminished dendritic cell function. J. Immunol. 181:746–755.
47. Matsuda, M., et al. 1998. Decreased expression of signal-transducing CD3 zeta chains in T cells from the joints and peripheral blood of rheumatoid arthritis patients. Scand. J. Immunol. 47:254–262.
48. Maurice, M. M., et al. 1997. Defective TCR-mediated signaling in synovial T cells in rheumatoid arthritis. J. Immunol. 159:2973–2978.
49. McGuire, K. L., and M. Iacobelli. 1997. Involvement of Rel, Fos, and Jun proteins in binding activity to the IL-2 promoter response elements in human T cells. J. Immunol. 159:1319–1327.
50. Miyara, M., et al. 2006. The immune paradox of sarcoidosis and regulatory T cells. J. Exp. Med. 203:359–370.
51. Moller, D. R. 2003. Treatment of sarcoidosis—from a basic science point of view. J. Intern. Med. 253:31–40.
52. Moller, D. R. 2007. Potential etiologic agents in sarcoidosis. Proc. Am. Thorac. Soc. 4:465–468.
53. Nambari, M. P., et al. 2001. Dexamethasone modulates TCR zeta chain expression and antigen receptor-mediated early signaling events in human T lymphocytes. Cell. Immunol. 208:62–71.
54. Newman, L. S., et al. 2004. A case control etiologic study of sarcoidosis: a current perspective. Arch. Intern. Med. 164:374–381.
55. Otsuji, M., et al. 1997. Changes in expression of signal transduction proteins in binding activity to the IL-2 promoter CD28-responsive element by NF-ATp and various NF-kappaB/Rel transcription factors. Mol. Cell. Biol. 17:2605–2614.
56. Pandiyam, P., et al. 2004. CD152 (CTLA-4) determines the unequal resistance of TH1 and TH2 cells against activation-induced cell death by a mechanism requiring PI3 kinase function. J. Exp. Med. 199:831–842.
57. Parker, D. C. 1995. T cell-dependent B cell activation. Annu. Rev. Immunol. 13:331–360.
58. Paz Morante, M., et al. 2006. Activation-associated phenotype of CD3 T cells in acute graft-versus-host disease. Clin. Exp. Immunol. 145:36–43.
59. Sallusto, F., J. Geginat, and A. Lanzavecchia. 2004. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu. Rev. Immunol. 22:745–763.
60. Scheinman, R. I., P. C. Cogswell, A. L. Lofoquist, and A. S. Baldwin, Jr. 1995. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. Science 270:283–286.
61. Schubert, L. A., et al. 1995. The human gp39 promoter. Two distinct nuclear factors of activated T cell protein-binding elements contribute independently to transcriptional activation. J. Biol. Chem. 270:29624–29627.
62. Sica, A., et al. 1997. Interaction of NF-kappaB and NFAT with the interferon-gamma promoter. J. Biol. Chem. 272:30412–30420.
63. Smith-Garvin, J. E., G. A. Koretzky, and M. S. Jordan. 2009. T cell activation. Annu. Rev. Immunol. 27:591–619.
64. Swiss, N. J., et al. 2010. Significant CD4, CD8, and CD19 lymphopenia in peripheral blood of sarcoidosis patients correlates with severe disease manifestations. PLoS. One 5:e9088.
65. Vallabhapurapu, S., and M. Karin. 2009. Regulation and function of NF-kappaB transcription factors in the immune system. Annu. Rev. Immunol. 27:693–733.
66. Van Laethem, F., et al. 2001. Glucocorticoids attenuate T cell receptor signaling. J. Exp. Med. 193:503–514.
67. Wahlstrom, J., et al. 1999. Phenotypic analysis of lymphocytes and monocytes/macrophages in peripheral blood and bronchoalveolar lavage fluid from patients with pulmonary sarcoidosis. Thorax 54:339–346.
68. Well, R., and A. Israel. 2004. T-cell receptor- and B-cell receptor-mediated activation of NF-kappaB in lymphocytes. Curr. Opin. Immunol. 16:374–381.
69. Winterbauer, R. H., and K. G. Kraemer. 1976. The infectious complications of sarcoidosis: a current perspective. Arch. Intern. Med. 136:1356–1362.
70. Wong, H. K., G. M. Kammer, G. Dennis, and G. C. Tsokos. 1999. Abnormal NF-kappa B activity in T lymphocytes from patients with systemic lupus erythematosus is associated with decreased p65-ReIA protein expression. J. Immunol. 163:1682–1689.
71. Yamamoto, R., et al. 2008. PD-1/PD-1 ligand interaction contributes to immunosuppressive microenvironment of Hodgkin lymphoma. Blood 111:3220–3224.
72. Younes, S. A., et al. 2003. HIV-1 viremia prevents the establishment of interleukin-2-producing HIV-specific memory CD4+ T cells endowed with proliferative capacity. J. Exp. Med. 198:1905–1922.
73. Zajac, A. J., et al. 1998. Viral immune evasion due to persistence of activated T cells without effector function. J. Exp. Med. 188:2205–2213.
74. Zea, A. H., et al. 1998. Changes in expression of signal transduction proteins in T lymphocytes of patients with leprosy. Infect. Immun. 66:499–504.