The new guideline for BAL cellular analyses is, in fact, in keeping with the evidence-based 2011 guidelines for diagnosis and management of IPF. Acknowledging the observations by Ohshimo and colleagues (3) as noted by Wuyts and colleagues, the 2011 guidelines addressed this concern and provided specific remarks regarding this (2). Furthermore, Ohshimo and coworkers used the criteria from the 2002 American Thoracic Society/European Respiratory Society Consensus Classification for making a diagnosis of IPF in the absence of a surgical lung biopsy (4), not the recommendations given in the more current 2011 IPF consensus guideline for the diagnosis and management of IPF. Because the question regarding the usefulness of BAL cellular analyses in patients with suspected IPF had been addressed and remarked upon in the published IPF guideline in 2011, we simply had alerted readers to the recommendations and comments in the 2011 IPF guidelines that pertained to the utility of BAL in making a diagnosis of IPF. We also suggested that with very advanced, end-stage pulmonary fibrosis, BAL cellular analysis is unlikely to be diagnostically useful, but we do not suggest that BAL should not be used to investigate less severe ILD.

Wuyts and colleagues referred to the old BAL guideline published by Haslam and Baughman in 1999 (5) regarding the need to segregate the first 20-ml aliquot and not pool it with subsequent, pooled aliquots used to assess the BAL cell profile. The present committee (including Dr. Baughman) reviewed all evidence to date (as well the old guidelines) and could not find ample evidence to justify a need to segregate the first aliquot for routine clinical analyses. If segregation of the first aliquot is done versus pooling of all aliquots for BAL cellular analysis, the method should be stated in reports of BAL analysis and in publications.

BAL can be a very useful diagnostic procedure to assist in the diagnosis of ILD, but it must be considered along with appropriately collected and comprehensive clinical data, adequate thoracic imaging, and tissue biopsy (if obtained). Expert chest radiologists can often reach a highly likely ILD diagnosis (other than a confident UIP pattern) based on interpretation of current, state-of-the-art HRCT imaging, and BAL cell patterns (when BAL is properly obtained and processed and BAL cell patterns are properly interpreted) can be used to support specific non-IPF ILD diagnoses (6).

Finally, Wuyts and colleagues express the concern that guidelines should be intended to be read by "nonexpert readers." It is hoped that the nonexpert reader will become educated by keeping up with new evidence published in the medical literature. The main object of providing new guidelines that are sponsored by professional societies is to provide the nonexpert reader with guidelines supported by available and newer evidence and/or by consensus among a panel of experts when evidence is poor or nonexistent with the hope that clinicians will adopt and/or change their clinical practice accordingly with the goal of benefitting patients. It is our hope that clinicians confronted with clinical management of patients will educate themselves and change their clinical practices by examining new evidence and guidelines rather than stepping back and continuing to follow guidelines of the same topic published over a decade ago.

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Comments on the Neonatal Bacillus Calmette-Guérin Vaccination: Adding Notes in Proof of Nonspecific Effect

To the Editor:

We read with interest the article by Ritz and colleagues (1) that found that early vaccination using three related bacillus Calmette-Guérin (BCG) strains induces dissimilar patterns in children's *in vitro* cellular immunity. Also, it should be noted that mounting evidence for the rapid induction of innate immune responses by BCG may protect neonates from infection due to expression of perforin and granulysin by both natural killer and T cells from cord blood (2). However, we were quite surprised by the fact that apparently normal immune function could be detected in that *in vitro* study (1).

Regarding this latter topic, we would like to comment on important published (3) as well as unpublished data. When assaying cord blood cells in parallel with otherwise healthy adult peripheral blood mononuclear cells in Brazil, we uncovered significant information concerning not only innate but also adaptive immune responses in neonates vulnerable population. Our previous findings (3) support the hypothesis that BCG induces distinct cell-death patterns involving maturation of the immune system and that these patterns might set the stage for a subsequent antimycobacterial immune response, which may induce profound effects during vaccination. However, this hot topic was not suitably addressed or commented upon by the above-mentioned group (1). Image and frequencies (appended table) of IFN-γ enzyme-linked immunospot (ELISPOT) responses at the single-cell level and expressed as the number of spot-forming colonies for two *Mycobacterium tuberculosis* RD1–specific antigens (ESAT-6 and CFP-10), as well as three shared *M. tuberculosis* and *M. bovis* BCG antigens (Ag85A, Ag85B, and hsp65) tested in two adults and two neonates are shown in Figure 1. The *in vitro* Th1-immune response of neonates was deficient when cells were placed in contact with those recombinant antigens. In fact, the probable impairment was related to a nonspecific immune response, because the potent mitogen phytohemagglutinin (PHA) assayed in parallel as an internal positive control yielded virtually no response in that group. A potential artifact was ruled out because cells from

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vaccinated adult individuals in matching identical assays yielded convincing data (Figure 1). The reasons behind this result are speculative; it may be due to a higher amount of circulating immature immune cells or to a lack of exposure to mycobacterial antigens. Actually, because of decreased Th1 cell–associated cytokine production, it is thought that the neonatal innate immune system is generally impaired or depressed. The bias against Th1 cell–polarizing cytokines leaves newborns susceptible to microbial infection, as reviewed and pointed out by Levy (4), and contributes to impairment of neonatal immune responses to most vaccines (5). The ability of proinflammatory cytokines to induce spontaneous abortion is likely to be an important reason for the strong bias of the maternal and fetal immune systems of many mammalian species toward Th2 cell–polarizing cytokines (4). After birth, there is an age-dependent maturation of the immune response. In our model, the readout was possible using the U.S. Food and Drug Administration– and CDC-licensed, certified immunodiagnostic platform of T-SPOT.TB (Oxford Immunotech Inc., Oxford, UK), with minor modifications (BCG antigens are not part of the commercial kit). Also, it is important to bear in mind that the peripheral blood-derived IFN-γ response has been extensively investigated using both T-SPOT.TB and QuantiFERON-TB (Cellestis, Carnegie, Australia) for the management of adult and child tuberculosis but has never been tested using umbilical vein cells from naive neonates. Our ELISPOT data not only support the above report (3) but also add another round of information regarding the immune response against mycobacteria in a population prone to receive the BCG vaccination after birth. This is particularly interesting based on the current policy of Guinea-Bissau to delay that immunization protocol in low-birth-weight infants (6). Somebody else could assay cord blood cells using the model proposed here in cohorts of children by the time BCG vaccination takes place. In addition, the important topic of the triggering of innate and adaptive immune responses by vaccines deserves further study. Therefore, follow-up trials are warranted to better clarify this central issue.

Part of this work was presented at the 2009 and 2010 Keystone Symposia (7, 8).

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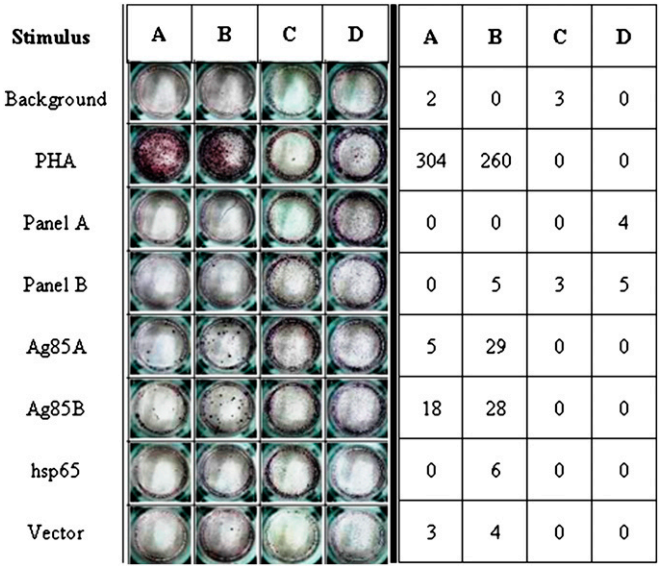

Figure 1. IFN-γ production evaluated by a commercial enzyme-linked immunospot assay (T-SPOT.TB with minor modifications) in primary peripheral blood mononuclear cell cultures using phytohemagglutinin (PHA), *Mycobacterium tuberculosis* RD1–specific ESAT-6 (Panel A) and CFP-10 (Panel B) antigens, and *M. bovis* bacillus Calmette-Guérin (BCG) Ag85A, Ag85B, and hsp65 antigens in healthy adult mononuclear (columns A and B) and neonate cord blood (columns C and D) cells. PHA and BCG antigens were used at final concentrations of 1% and 5 μg/ml, respectively. Numbers in the table show the spot-forming colonies, and "Vector" means the internal negative control (empty plasmid for the BCG antigens).

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Reply: Bacille Calmette-Guérin Vaccine: Innate Immunity and Nonspecific Effects

From the Authors:

We thank Ponte and colleagues for their interest in our study that compared the immune response to different bacille Calmette-Guérin (BCG) vaccine strains given at birth (1). We agree that the neonatal immune response differs both quantitatively and qualitatively from that later in infancy and childhood. We, and others, have previously reported that mitogen-induced cytokine production is dependent on age with lower concentrations of interferon (IFN)-γ measured in response to phytohemagglutinin (PHA) in infants and young children (2). This results in a high proportion of indeterminate results in IFN-γ release assays, particularly in children under the age of 5 years, and is one of the limitations for the use of IFN-γ release assays in young children (3).

Ponte and colleagues express surprise that we were able to detect PHA-induced responses in our study, particularly as, using an enzyme-linked immunosorbent spot assay, they did not detect IFN-γ production in PHA-stimulated cord blood from two newborns.