Reviewer's report

Title: Identification of a predominant isolate of Mycobacterium tuberculosis using molecular and clinical epidemiology tools and in vitro cytokine responses.

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Version: 1 Date: 31 Dec 2002

Reviewer: Dr Rogelio Hernandez Pando

Level of interest: not specified

Advice on publication: Other (see below)

This is an interesting study which try to demonstrate that one specific M. tuberculosis isolate from a specific geographic area in Canada is hypervirulent, by using epidemiological data and in vitro cytokine production by one infected human monocyte cell line. As was commented by the authors in the paper this point should be better demonstrated by in-vivo experiments using an animal model. Some relevant information and determinations are missing.

1.- In the introduction section it is important to add more information about the aborigen group from which the strain 1 was isolated. What kind of ethnic group is?. Why this isolate is so prevalent in this human group?. What kind of diet they have?. Is common malnutrition, alcoholism or diabetes in this ethnic group or what kind of another predisposition factor for tuberculosis susceptibility is present in this human group.

2.- In the material and methods section it is important to mention the number of in vitro passages that the different strains have, because it is well known that many passages in culture medium usually decrease virulence. Also is important to know the curve rate in the culture medium of the different M. tuberculosis strains, and from which part of the growth curve the bacteria were obtained for in-vitro monocyte infection. Ideally the different mycobacteria strains should be synchronized in the in-vitro growth and gotten the same phase of the growth curve in order to get comparable results of the monocyte infection experiments, considering that bacilli obtained from the log phase are metabolically different than organisms obtained from the stationary phase, bacteria from these different growth phases could give a different pattern of macrophage cytokine production, and the growth rate into the cytoplasm of infected cells could be also different.

3.- The most important cellular source of IFNgamma are T lymphocytes, thus the low IFNgamma production by monocytes infected by strain 1 is less important that the IFNgamma production by T cells in mixed cell culture experiments. This point should be mentioned in the text. Macrophages are not a significant source of IL-4, again this is a lymphocyte cytokine, so the IL-4 determination was irrelevant and should be discarded. If it is possible, it will be important to measure TGFbeta in the supernatants by ELISA, this is another extremly important anti-inflammatory cytokine in mycobacterial infection and their most important source are macrophages.
Competing interests:
None declared.