行政院國家科學委員會補助專題研究計畫成果報告

探討肺泡第二型細胞在高氧肺傷害之病理機轉

計畫類別：■個別型計畫　□整合型計畫
計畫編號：NSC89－2314－B－002－076－
執行期間：88年08月01日至90年07月31日

計畫主持人：廖永祥
共同主持人：

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- □赴國外出差或研習心得報告一份
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- ☑出席國際學術會議心得報告及發表之論文各一份
- □國際合作研究計畫國外研究報告書一份

執行單位：國立台灣大學醫學院內科

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一. 中英文摘要

本計劃原本探索在高氧刺激下，肺泡細胞產生或減少的基因變化，藉此闡明高氧肺傷害對於對於肺泡細胞可引發或抑制不同的基因，進而瞭解其分子及細胞的詳細機轉；當完成肺泡細胞利用顯微螢光光譜術測量細胞內酸鹼變化及AE的分子生物cloning後，個人卻罹患肝疾，歷經兩次手術及其他相關治療，在家休養14個月後，原本建立實驗裝置及細胞培養，因時空轉移及客觀因素皆已改變，所欲探討高氧肺傷害所成肺泡細胞之反應機轉亦有文獻發表。經由指導教授同意後，修改研究方向，探討NRAMP1在台灣族群的表現。NRAMP1與巨噬細胞吞噬體之酸化作用有關，與本人多年研究肋膜細胞酸化調控機轉相關連。本實驗共研究48位正常人，49位結核病患，60位愛滋病患，抽取gemonic DNA分析NRAMP1變異性：在研究樣本中A318V不具變異性，274C/T及577-18G/A絕大多數為C/C及G/G; 只有D543N及1729+55del4具有較大變異性。然而D543N及1729+55del4變異性在這三組樣本中，並不具統計學之差異。最大的發現在與我們的基因表現與歐美人種不同，這樣的結果是否能解釋台灣及亞裔族群較易得到結核病則有待進一步研究。

The contribution of *Nramp1* to anti-tuberculosis resistance in human remains debated. Here we reported results using 5 genotypes (274C/T, 577-18G/A, A318V, D543N and 1729+55del4) in analyzing genetic polymorphism of *NRAMP1* in Taiwanese. The study sample enrolled 49 patients with tuberculosis and 48 healthy control subjects. We found A318V was not polymorphic in our population. Only D543N and 1729+55del4 showed more heterozygous. In 274 C/T and 577-18G/A, the allele frequencies showed the predominant type as homozygous pattern C/C (94%) and G/G (94%), respectively. There were no statistically significant differences between tuberculosis patients and healthy control subjects. In spite of the high susceptibility to *M. tuberculosis* in HIV-infected patients, the allele frequencies of D543N and 1729+55del4 in these patients were not significantly different between the HIV-positive patients (n=60) and the HIV-negative subjects (n=97).

Genotypic frequencies in the HIV-positive patients were not significantly different between the tuberculosis patients (n=29) and the non-tuberculosis patients (n=31). Compared to previous studies, we found significant differences among different racial groups in allele frequencies for 274C/T, D543N and 1729+55del4. In conclusion, the allele and genotype of *NRAMP1* polymorphism in Taiwanese were different from the Caucasians, Africans and Spanics. No allelic associations were identified among the *NRAMP1* alleles, tuberculosis susceptibility, and HIV infection.

二. 計畫緣由與目的

Tuberculosis is still a leading death of infectious diseases in Taiwan (Yu, 1999). Emerging evidences have suggested that genetic factors are involved in susceptibility of infection by pathogens (Liu J, 1995; Barton CH, 1994). It has generally been assumed that resistant of mice to infection with *M. tuberculosis* is determined, to a significant degree, by the action of *Nramp1*. Whereas, the contribution of *NRAMP1* to anti-tuberculosis resistance in human remains debated (North, 1998; Greenwood, 2000). Recent studies have found an association of *NRAMP1* with susceptibility to tuberculosis in a Gambian population and weak linkage in a family study (Bellamy RC, Ruwende T, 1998; Blackwell JM. 1998). Here we report the results using five genotypes in analyzing genetic polymorphism in Taiwanese with and those without tuberculosis.

三. 結果與討論

In order to establish the allele and genotype frequency of *NRAMP1* in Taiwanese, the first part of this study consists of 97 HIV-negative individuals, including 49 active tuberculosis and 48 healthy subjects. From analysis of 5 genotypes of *NRAMP1* polymorphisms, only D543N and 1729+55del4 were polymorphic in this population of Taiwan. A318V was not polymorphic; the alleles 274 C/T and 577-18G/A show a predominant allele as homozygous pattern C/C (94%) and G/G (94%), respectively. In D543N, homozygous G/G and A/A are 70% and 4%, respectively; and heterozygous G/A is 26%. In 1729+55del4, homozygous TGTG/TGTG and del/del are 69% and 6%, respectively; and heterozygous TGTG/del is 25%. In A318V, either through a genetic shift or a natural selection, no T/C or T/T was found in our HIV-negative population.

The control subjects denied the tuberculosis history and had no evidence of pulmonary tuberculosis in the chest roentgenograms. By the Odds ratio analysis and chi-square test, we failed to find statistically significant differences between these two groups. Among TB group, there were no significant associations of the family history, disease severity, outcomes, recurrent rate, and drug-resistance status with D543N and 1729+55del4 polymorphism.

Based on only D543N and 1729+55del4 polymorphisms in our population, the second part of this study was done to determine D543N and 1729+del4 polymorphisms in the HIV infection patients with or those without tuberculosis. Because HIV infection in Taiwan remains largely a male disease, there was an excess of male in the group of HIV –positive patients (55 male and 5 female). That was significantly different from HIV-negative groups (P<0.05). Table V shows the allele and genotype frequencies of *NRAMP1* polymorphisms in HIV-infected patients and the non HIV-infected subjects. The allele frequencies of those two *NRAMP1* polymorphisms were not significantly different between the HIV-infected patients and the non HIV-infected population. In addition, genotypic frequencies were not significantly different between the tuberculosis patients and the non-tuberculosis patients. In the HIV-infected patients, we found D543N allele variant was frequently linkage disequilibria with 1729+del4.

Comparing to previous studies, we found that there were significant differences of allele frequencies between different racial groups, for 274C/T, D543N and 1729+55del4. Except for 274C/T, the allele frequencies of D543N and 1729+del4 between Taiwanese and the Hong Kong people were similar; the D543N allele frequency of Taiwanese and Hong Kong people is significantly different from Caucasians, Africans, and Colombia Spanics. Moreover, the 1729+55del4 allele frequency among Taiwanese, Hong Kong people, Africans and Colombia Spanics is different from that of Caucasians.

計畫成果自評

1. The allelic and genotype of *NRAMP1* polymorphism in Taiwanese were different from the Caucasians, Africans and Spanics. No allelic association was identified between the *NRAMP1* alleles and tuberculosis susceptibility. In addition, neither homozygotes nor heterozygotes for any *NRAMP1* variants were increased risk of HIV infection.
2. It is not clear whether this discrepancy is due to an ethnic difference or a genetic advantage (or disadvantage). Although the variant alleles of D543N and 1729+55del4 were not associated with tuberculosis in this study, they would be possible in linkage disequilibria with another unknown functional polymorphism. A new *sst1* gene (susceptibility to tuberculosis 1), 10-19 centimogran distal to *NRAMP1*, has been reported to control progression of tuberculosis infection in a lung-specific manner (Kramnik, 2000). The linkage of *sst1* and *NRAMP1* needs to be further elucidated.
3. This result has been submitted to the International Journal of Tuberculosis and Lung Disease.

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出國開會心得報告

廖永祥

5月18日接受國科會補助前往美國舊金山參加爲期六天的「美國胸腔病學2001年世界年會」，當一下飛機時，百感交集，因個人健康因素，我已連續3年未參加此盛會，望著碧藍的天空，美麗的高樓天際線，雄偉的舊金山大橋，各式各樣的高樓，勾起了1997年至此開會的回憶，能重遊故地，真是感謝神的恩典。此一次盛會共有一萬五千多人參加，包括胸腔臨床醫師、呼吸治療師、小兒科呼吸道專家醫師及基礎醫學研究學者。藉此盛會，使全世界各地學術、研究有互相交流的機會，了解別人正在從事哪些研究工作，知己知彼，方能避免重覆別人已發表之研究。這一次我著重在參加結核病的各種研討會及論文發表會，因爲台灣的結核病的基礎研究，亦是一件刻不容緩的事情。如同第一天在「Management of Tuberculosis:Advanced Practice」主席UCSF Hopeorell教授所說的「全球性的結核病所造成的罹病及死亡的程度，讓人觸目驚心又無法忍受，這個全球性的問題不佳是健康上重大的威脅；在成人及小孩的有效治療必須是第一線治療整合成一部分，藉著直接觀察療法，在2005年時，能達到70%新的結核病選擇及治療必須每一個國家都重視結核病的嚴重性及急迫性，採取有效的治療，才能有效達到全球性結核病的防治工作。在全世界如此重視結核病的研究及防治，台灣更不能置身於世界之外。整個會程中，所討論的題目包括，流行病學研究、分子生物在結核病之研究，潛伏性結核病的治療，抗藥性結核病治療之新趨勢，結核病與愛滋病的關係。在第二天的海報展示中，我報告了Nrampl基因在台灣族群的表現及與結核菌的關聯性。吸引了不少人的注意，而大多數對於我們的基因表現與歐美人種不同，感到興趣，希望以後能有跨國的研究，總而言之，六天的議程，讓我收穫滿滿，藉著此次充電的機會，希望對台灣結核病的基礎研究能有些貢獻。