

TUBERCULOSIS (TBC) is characterized by a complex immune response which parallels the clinical course of the disease. In this respect, acquired resistance, delayed hypersensitivity reaction and anergy are the main types of immune reactivity to mycobacterial antigens. In view of the presence of nonspecific and specific immune deficits in TBC patients, a clinical trial was carried out in a group of 20 individuals with active pulmonary TBC by oral administration of acetyl-L-carnitine (ALC). This drug, which has been shown to possess immunomodulating activities, was able to upregulate the T-dependent antibacterial activity in TBC patients after 30 days' treatment, while the same activity decreased in patients receiving placebo only. On the other hand, ALC did not modify serum levels of tumour necrosis factor- $\alpha$ , in the same individuals. This cytokine plays a detrimental rather than beneficial role in TBC pathogenesis. In the light of these data, ALC seems to be a powerful immunomodulator in the course of *Mycobacterium tuberculosis* infection and other mycobacteriosis.

**Key words:** Acetyl-L-carnitine, Immune response, Lymphocytes, Macrophages, Tuberculosis, Tumour necrosis factor- $\alpha$

## Immunological responses in patients with tuberculosis and *in vivo* effects of acetyl-L-carnitine oral administration

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## Introduction

Tuberculosis (TBC) is a pathological process characterized by a broad spectrum of immunological responses which correlate with the clinical course of the disease.<sup>1–3</sup> In TBC, immune responsiveness depends on three basic mechanisms: (1) acquired cellular resistance; (2) delayed type hypersensitivity (DTH); and (3) anergy.<sup>1–3</sup>

Acquired cellular resistance is a protective mechanism in which specific T-cells are activated by macrophages, the so-called antigen presenting cells.<sup>4</sup> With special reference to mycobacterial antigens, mainly proteins, e.g. heat shock proteins (HSP), are presented by macrophages to both human and murine  $\gamma\delta^+$  cells.<sup>5–8</sup> On the other hand, murine CD4<sup>+</sup> and CD8<sup>+</sup> cells seem to participate to acquired resistance,<sup>9</sup> even if others claim that only CD4<sup>+</sup> cells are the major protective subset in long-term protective studies.<sup>10</sup> In humans, recent studies have pointed out that CD4<sup>+</sup> cells kill monocytes which contain mycobacteria regardless of the T-helper (h)<sub>1</sub>-Th<sub>2</sub> subset distinction and of the HSP recognition.<sup>11</sup>

DTH is a detrimental reaction dependent on the sensitization of specific lymphocytes by mycobacterial antigens and usually leads to granuloma formation.<sup>1–3</sup> Granuloma, which consists of an accumulation of macrophages, may undergo either a protective evolution with microorganism neutralization or a caseification with tissue damage.<sup>1–3</sup>

In the phase of macrophage accumulation, two inflammatory cytokines (CKs), namely the monocyte chemotactic protein-1 (MCP-1) and the macrophage inhibiting factor play an important role.<sup>1–3</sup> In the phase of tubercular granuloma evolution interferon- $\gamma$  (IFN- $\gamma$ ) and interleukin-2 (IL-2) are protective,<sup>12,13</sup> while tumour necrosis factor- $\alpha$  (TNF $\alpha$ ) is mostly involved in granuloma destruction.<sup>14</sup>

With special reference to TNF $\alpha$ , it has been reported that lipoarabinomannan (LAM) from the *M. tuberculosis* cell wall is responsible for the production of this CK,<sup>15</sup> as is also confirmed by studies on LAM-induced transcription of mRNA for several CKs, even including TNF $\alpha$ .<sup>16</sup> Furthermore, by analogy with other intracellular infections (e.g. *M. avium* and *M. bovis* infections, listeriosis, toxoplasmosis and trypanosomiasis)<sup>17</sup> TNF $\alpha$  should also play a protective role in the case of *M. tuberculosis* infection. However, according to Filley and Rook<sup>18</sup> virulent TBC strains produce a factor which alters the normal function of TNF $\alpha$ , rendering it toxic to host tissue. Therefore, many clinical and laboratory findings in TBC patients may be ascribed to TNF $\alpha$  release, such as fever, weight loss, necrosis, intravascular coagulation and acute phase protein release.<sup>19</sup> On the other hand, data of Barnes *et al.*<sup>20</sup> support a protective role for TNF $\alpha$  in the course of pleuritis, which usually resolves in the absence of therapy. Moreover, Takashima *et al.*<sup>21</sup> report a reduction of TNF $\alpha$  in patients with chronic

TBC. Taken together, these findings attribute a noxious role to TNF $\alpha$  in the course of TBC, while beneficial effects of this CK may arise when it is moderately produced or locally released as in the case of pleural effusion.

Anergy is a clinical condition of depressed immune function, as observed in patients with advanced refractory TBC.<sup>1-3</sup> Several mechanisms have been postulated for explaining the immunosuppression in TBC patients. In this respect, bacterial cell wall components, e.g. D-arabino-D-galactan, can downregulate Fc receptor (R) function on cell membranes by circulating in the form of immunocomplexes.<sup>22</sup> Furthermore, prostaglandin E<sub>2</sub> release by monocytes may reduce IL-2 production and decrease expression of IL-2 R.<sup>23</sup> Another immunosuppressive agent is represented by IL-1, which inhibits T-lymphocyte proliferation by protein purified derivative (PPD) when produced in exaggerated amounts.<sup>24</sup> Finally, a 25 kDa glycoprotein derived from *M. tuberculosis* has been shown to inhibit phagocytic functions.<sup>25</sup>

These data emphasize that a plethora of suppressive factors may be involved in the depression of immune response in TBC patients and their recognition is important for the establishment of therapeutic strategies.

### Monitoring of the Immune Status in Patients with TBC

Until now, many studies have been focused on T-lymphocyte functions in TBC individuals and, in particular, T-cell response to mitogens and antigens has been evaluated. In contrast, less information is available on polymorphonuclear cell (PMN) and monocyte functions, and on B-cell activity.

In a recent study, a group of 14 patients with TBC (seven individuals with acute disease and seven individuals with chronic disease) have been evaluated in terms of phagocytic and B-cell functions.<sup>26</sup> PMN and monocyte activities were assessed by determining their chemotactic capacity and ability to phagocytose *Candida albicans*. At the same time, the release of two inflammatory CKs, leucocyte inhibiting factor (LIF) and MCP-1 was determined. Results show that chemotaxis, phagocytosis and killing were reduced regardless of the disease status.<sup>26</sup> The impaired release of LIF and MCP-1 could also explain the altered phagocytic activity.

Assessment of B-cell responsiveness was performed by evaluating *in vitro* antibody synthesis in a plaque-forming cell system, using pokeweed mitogen (PWM) as a polyclonal activator and PPD as a specific antigen. In patients with acute TBC, anti-PPD antibody response was significantly augmented, while in patients with acute and chronic

TBC PWM-induced antibody production fell within normal values.<sup>26</sup> These last results are in accordance with findings of others who detected elevated IgG serum levels to PPD in acute TBC.<sup>27</sup> Moreover, a strict correlation was found between ELISA positivity for anti-PPD IgG antibody and TBC diagnosis.<sup>28</sup> In chronic patients the lack of anti-PPD antibody response may depend on B-cell immunosuppression, despite an elevated frequency of B-cells.

The bulk of the above data support the usefulness of monitoring different compartments of the immune system in TBC patients, since deficits in these individuals seem to be more vast than expected.

### Oral Administration of Acetyl-L-carnitine in Patients with Active Pulmonary Tuberculosis

Much evidence supports the concept that the immune response plays an important role in the pathogenesis of TBC. Quite interestingly, the authors' previous results emphasize that even patients in the acute phase exhibit multiple deficits of either nonspecific or specific immunity. Now, the question arises concerning the opportunity to immunomodulate these subjects in order to avoid superimposition of other infections (e.g. historically, measles; nowadays, HIV infection). However, the risk exists that classical biological response modifiers (BRM), which enhance immune response via CK release,<sup>29</sup> may trigger detrimental DHRs for the host. In this framework, it was reasoned that a putative BRM, which may increase the metabolic performance of immune cells rather than inducing release of noxious mediators, should be more appropriate for treating TBC subjects.

Just recently, ALC, a substance which is actively involved in either the transport and oxidation of fatty acids into mitochondria, or in the production and incorporation of their unsaturated forms into membrane phospholipids,<sup>30</sup> has been shown to possess immunoregulatory properties. Studies in this direction have, in fact, demonstrated, that ALC reduced macrophage and lymphocyte function decline in aged rats<sup>31</sup> and enhanced mitogen-induced lymphocyte proliferation in the elderly.<sup>32</sup> In AIDS subjects treated with ALC an improvement of immunocompetence was found<sup>33</sup> and this correlates with the reduced levels of carnitine detected in these patients.<sup>34</sup>

On this basis, 20 subjects with active pulmonary TBC were enrolled in a double blind, randomized, controlled parallel study.<sup>35</sup> Ten patients were treated orally with 2 g ALC/day (Nicetile, Sigma-Tau, Pomezia, Italy) for 30 days. The control group received placebo only. Two main immune para-

meters were followed up at day 0 and day 30, namely the antibacterial activity and TNF $\alpha$  serum levels.

Antibacterial activity is a T-cell function dependent on CD4<sup>+</sup> and CD8<sup>+</sup> cell activity which represents a good index of the host resistance against pathogens, even including *M. tuberculosis*.<sup>36</sup> This activity was depressed in both groups of patients at time 0, but, while it increased in the ALC-treated individuals, it decreased in the subjects receiving placebo only. Several hypotheses can be formulated to explain the immunomodulating activities of ALC: (1) ALC may act by supplementation of energy to lymphocytes via ATP; (2) chemotherapy may impair lymphocytic functions in these patients<sup>37,38</sup> and ALC eventually potentiates the reduced antibacterial activity and/or prevents iatrogenic immunosuppression; or (3) ALC can modulate the hypothalamus-pituitary-adrenal axis with release of enhancing neurohormones and neuropeptides.<sup>39</sup>

As far as determination of serum levels of TNF $\alpha$  is concerned, no differences were seen between the two groups, since mean values of this CK fluctuated within normal ranges before and after treatment. However, in three cases within the ALC-treated group and in two cases within the placebo group TNF $\alpha$  levels increased by day 30. Although anamnesis, clinical examination, disease and state and other related blood parameters were taken into consideration, a reasonable explanation for understanding the above finding was not achieved. It is noteworthy that this present data confirms that reported by Rook *et al.*,<sup>19</sup> who also found normal serum levels of TNF $\alpha$  in TBC patients. In this context, to extend our data further, studies will evaluate *in vitro* TNF $\alpha$  release from LAM-stimulated monocytes in ALC-treated TBC patients. In fact, detection of TNF $\alpha$  in serum can be prevented by the presence of inhibitors found in TBC and sarcoidosis.<sup>40</sup>

### Therapeutic Application of Acetyl-L-carnitine in Mycobacteriosis

Tuberculosis and leprosy are the two major mycobacterial infections in which a deficit of Th cells has been recognized, mostly in terms of release of protective CKs, such as IL-2 and IFN- $\gamma$ .<sup>1,3,12,13,41,43</sup> On the other hand, according to Grau *et al.*<sup>44</sup> TNF $\alpha$  may cause both protection and clinical manifestations in mycobacterial disease and pathology should be interpreted as the consequence of the protective mechanism. Therefore, a pharmacological modulation of the immune response in mycobacteriosis should be applied to the T-cell compartment, excluding the monocyte-macro-

phage system whose products, e.g. IL-1 and TNF $\alpha$ , are more detrimental than beneficial.<sup>24,44</sup>

The authors' data indicate that ALC may represent a suitable BRM for treating mycobacteriosis, since it increases T-cell dependent antibacterial activity without effect on *in vivo* TNF $\alpha$  production in the affected patients. In this respect, the improvement of clinical and immunological findings observed in ALC-treated AIDS patients<sup>33</sup> indirectly confirms that this compound acts on lymphocytes but not on cellular sources of TNF $\alpha$ . In fact, several papers support the prominent role of TNF $\alpha$  as the cofactor in the triggering and worsening of HIV infection.<sup>45,48</sup>

Finally, even if these preliminary results seem to support the immunomodulating capacities of ALC, long-term studies and monitoring of additional immune parameters are needed on a broader sample of the population affected by mycobacterial disease.

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