Administration of an Autogenous Vaccine in Patients with Chronic Bacterial Osteomyelitis

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Abstract  Since we face the problem of rapidly growing rates of antimicrobial resistance, autovaccination may provide a treatment alternative at least in those patients which suffer from treatment refractory infections. Interest is turning towards the therapy of infectious diseases by stimulation of the immune defence mechanisms. In fact there are reports of drug resistance in a wide range of bacterial diseases. In our experience, autovaccine immunization has the potential to treat chronic infections such as osteomyelitis unresponsive to antimicrobial therapy.

Keywords  Osteomyelitis, Autovaccine, Antimicrobial Agents

1. Introduction

Autogenous vaccination was a well accepted method for the treatment of infectious diseases in the first half of the 20th century.

Following invention of antimicrobial agents, autovaccines lost their importance in human medicine.

The principle of homologous autovaccination is to isolate the disease causing microorganism from the site of infection. [1-3]

Following growth to pure culture, the organism is inactivated to produce an inactivated whole cell vaccine which is given to the patient in several intervals.

Osteomyelitis is a disease characterized by an infection of either the cortex or the medullar portion of the bone.

It is obvious that chronic osteomyelitis is still a serious medical problem and that newer forms of therapy must be sought and investigated.

Among the aetiologic agents responsible for osteomyelitis, both gram-positive and gram-negative bacteria play an important role.

In particular, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Serratia marcescens are the most frequent strains isolated from infected bones [4].

This disease frequently undergoes a chronic clinical course and is poorly responsive to chemotherapeutic agents [5].

On the other hand, the possibility that an impaired immune response might account for the development of osteomyelitis has been not yet extensively investigated [6].

In the present study, in Patients with chronic osteomyelitis, polymorphs (PMN) and monocytes phagocytosis and killing, leukocyte inhibitory factor (LIF) release and B cell function have been assessed.

At the same time, from our previous experience, an autogenous vaccine was prepared and administered for these subjects, in which immune parameters have been revaluated at different intervals of time following the immunotherapeutical regimen.[3, 7-8]

2. Materials and Methods

This study was conducted according to the recommendations of the Declaration of Helsinki; all Patients received informed consent.

Patients: For this study, 132 Patients with chronic osteomyelitis admitted in the Orthopedic Clinic of the University of Bari “Aldo Moro”, Medical School, have been evaluated.

Bacteria isolation: The following strains have been isolated from the Patients: Staphylococcus aureus, and Staphylococcus epidermidis.

Autogenous vaccine preparation: From each patient, the specific strain has been used for the vaccine preparation.

Briefly, bacteria have been cultured on brain heart-infusion agar and killed with formaldehyde.

After bacterial count and dilution, the vaccine has been injected for 10 days every second day.

Three additionally aliquots of this vaccine have been administered every month [7,8].

Phagocytosis and killing: PMN and monocyte have been evaluated for their capacity to engulf and digest specific bacterial strains according to the method of Mandell and Hook [9].

LIF assay: LIF release following stimulation of
lymphomonocytes with Concanavalin A (Con A) has been assessed in agarose plates according to Clausen [10].

3. Results

Non-specific and specific immune responses have been evaluated in 132 Patients with chronic osteomyelitis before vaccine treatment.

Table 1 shows that 114 patients exhibited a decrease of PMN and monocyte phagocytosis, while LIF activity was also significantly reduced, in comparison with normal controls. Only in 18 patients these values fell within normal ranges. On the other hand, number of spots for the assessment of B cell response was negligible.

Table 2 expresses the phagocytosis and LIF activity after first cycle of vaccination in 78 patients (no-responder) with a depressed immune response and in 18 patients with normal immune response (responders). Data shown an increase in both phagocytosis and LIF in the no responder group.

Table 3 illustrates the follow-up of antibody spot forming-cells during the course of vaccination. Quite interestingly, the increase of spots reached the maximum at forming-cells during the course of vaccination. Quite interestingly, in 12 Patients who underwent a relapse, either phagocytosis or LIF activity were dramatically decreased in comparison with values obtained before relapse.

Finally, Table 4 demonstrates that in 18 patients who underwent a relapse, either phagocytosis or LIF activity were dramatically decreased in comparison with values obtained before relapse.

Table 1. Immune status in 132 patients with chronic osteomyelitis before vaccine administration.

<table>
<thead>
<tr>
<th>Patients Phagocytosis* LIF* *</th>
<th>PMN</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>114 No-responders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 Responders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60.6±19.1°</td>
<td>52.6±11.7°</td>
<td>30.5±9.32°</td>
</tr>
<tr>
<td>87.0±3.2</td>
<td>87.6±5.3</td>
<td>54.3±2.4</td>
</tr>
</tbody>
</table>

Table 2. Immune responsiveness in 96 patients with chronic osteomyelitis at the end of vaccine administration.

<table>
<thead>
<tr>
<th>Patients Phagocytosis * LIF**</th>
<th>PMN</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N°</td>
<td></td>
<td></td>
</tr>
<tr>
<td>78 No-responders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 Responders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72.4±12.4±</td>
<td>70.4±8.9±</td>
<td>30.6±8.9</td>
</tr>
<tr>
<td>(55.7±19.1)*</td>
<td>(57.7±13.9)</td>
<td>(25.8±8.1)</td>
</tr>
<tr>
<td>85.3±6.1</td>
<td>85.2±4.7</td>
<td>60.3±11.2</td>
</tr>
<tr>
<td>(87.0±3.1)</td>
<td>(87.6±5.3)</td>
<td>(54.3±12.4)</td>
</tr>
</tbody>
</table>

Table 3. Evaluation of antibody spot-forming cells in 96 patients with chronic osteomyelitis before and after vaccine administration.

<table>
<thead>
<tr>
<th>Period</th>
<th>PMN</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>No-detectable</td>
<td></td>
</tr>
<tr>
<td>First cycle</td>
<td>106.6±57.1</td>
<td>319.1±27.4</td>
</tr>
<tr>
<td>Second cycle</td>
<td>252.1±18.8</td>
<td></td>
</tr>
<tr>
<td>Third cycle</td>
<td></td>
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</tbody>
</table>

* Values express the number of spots that indicate the reactivity of B cells with specific bacteria, antibody production is revealed by a peroxidase-anti-peroxidase system.

Table 4. Immune parameters in 18 patients with chronic osteomyelitis who underwent a relapse.

<table>
<thead>
<tr>
<th>Period</th>
<th>PMN</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before relapse</td>
<td>58.6±12.3±</td>
<td>54.0±5.4±</td>
</tr>
<tr>
<td>On relapse</td>
<td>60.6±17.1</td>
<td>16.6±5.3±</td>
</tr>
</tbody>
</table>

* Phagocytosis was evaluated as percent of cells that have engulfed bacteria.
* LIF was calculated in percent ci migration inhibition.
** Values are statistically significant before relapse (p<0.05).

4. Discussion and Conclusions

It is know that chronic osteomyelitis represents a serious medical problem, despite the use of effective surgical treatment or antibiotics and then newer forms of therapy must be investigated.

From our data the following points arise:

1) in the majority of Patients an impairment of either non-specific or specific immunity has been demonstrated, as also described in other chronic infections.

2) Clinical evaluation of Patients shows an improvement of the symptomathology (i.e. healing of fistulas and decrease of pain) in some subjects only.

3) Absence of side effects i.e. fever, hypotension, blood coagulation disorders etcetera.

Concerning the possible mechanisms of action of the autogenous vaccine, one can hypothesize that cell wall components such as lipopolysaccharides from gram-negative bacteria or muramyl-dipeptide from gram-positive organisms may positively modulate the antibody response. [11]

Additionally, one has to consider that in chronic osteomyelitis bacteria occupy a cryptic location in the bone with less exposure of their antigenic determinants to immunocompetent cells.

Quite interestingly, in 12 Patients who underwent a relapse, decreases of phagocytic function and LIF release are evident.

These findings reinforce the role of non-specific and specific immunity in the progression of chronic osteomyelitis.

In progress experiments are evaluating the effects of immunomodulating agents on the immunoresponsiveness in these Patients.

Surgical procedures done during the period of immunization and soon thereafter must also be considered as contributing to remission of the disease.
However, it is the impression of the attending Clinician that Patients treated with the vaccine probably benefited from the therapy.[12]

These data suggest that autovaccine represent an useful mean to improve the clinical and immunological parameters in chronic osteomyelitis.

**Acknowledgement**

We would like to dedicate this manuscript to honor the memory of Prof. Giovanni Rizzo, M.D., M.P.H.

**REFERENCES**


