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Mycobacterium Vaccae Vaccine Improve Incomplete Immune Recovery in AIDS Patients

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Abstract

Background: Mycobacterium seedling is rich in co-antigens and, a good immunomodulator with bidirectional immunomodulatory function.
Objectives: The present study aimed to explore whether the *Mycobacterium vaccae* vaccine can improve the incomplete immune recovery of acquired immunodeficiency syndrome (AIDS) patients.
Methods: According to the number of patients receiving human immunodeficiency virus (HIV) long-term treatment in the Ninth People's Hospital of Chongqing, China, 100 patients with HIV-RNA quantitation of less than 103 cepies/ml, CD4 + T cell count less than 500/UL, and $CD4^+$T cell count of more than 50/UL after 3 years of Haart treatment were selected. In total, they were randomly assigned to one of three groups, one group received an intramuscular injection of *M. vaccae* vaccine, one group received an intramuscular injection of growth hormone, and the last group received only a placebo injection. The levels and changes of naive T cells, Treg cells, total $CD4^+$ T cells, T helper 1 (Th1), T helper 2 (Th2) cells, and interleukin 7 (IL-7) cytokine were measured at baseline and after 12, 48, and 50 weeks.
Results: After 48 weeks of treatment, the total CD4+T cells, Th1 cells, and naive T cells in AIDS patients increased, while IL-7 cytokines, Treg cells, and Th2 cells decreased in *M. vaccae* vaccine and growth hormone group. There was no change in the placebo group, indicating that *M. vaccae* vaccine and growth hormone can improve the immune function of AIDS patients. After treatment, in group A (*M. vaccae* vaccine), there was an increase in the total $CD4^+$T cells, Th1 cells, and naive T cells as well as a decrease in Treg cells, Th2 cells, and IL-7 cytokines. The only significant difference between *M. vaccae* vaccine and growth hormone groups was a decrease in Th1 cells in the *M. vaccae* vaccine group. After a 12-week follow-up, it was found that there were no cases of secondary infection in the *M. vaccae* vaccine group, one case in the growth hormone group, and five cases in the placebo group, demonstrating that the secondary infection rate in *M. vaccae* vaccine and the growth hormone groups was lower than that in the control group.
Conclusion: The *M. vaccae* vaccine may decrease the levels of IL-7 as well as Treg and Th2 cells by increasing the levels of $CD4^+$T cells, Th1 cells, and pure T cells in AIDS patients, leading to the improvement of immune reconstitution of AIDS patients to some extent.

Keywords: AIDS, Immune reconstitution, Mycobacterium vaccae

1. Background

Acquired immunodeficiency syndrome (AIDS), a human syndrome characterized by immune deficiency, is caused by the human immunodeficiency virus (HIV). At present, highly active antiretroviral therapy (HAART) is the primary treatment for AIDS. The HAART can improve the immunodeficiency function of AIDS patients, improve their life quality, and extend life by increasing the number of immune cells ($CD4^+$T cells) and decreasing the amount of viral replication in the body.

Results of our previous studies have revealed that the proportions of $CD8^+$ T and Treg cells in AIDS patients were significantly lower after HAART treatment (1). However, there are specific differences between individuals. It has been reported that in approximately 20% of patients receiving viral inhibition (<50 copies/mL), the number of $CD4^+$T cells in the body did not increase, and immune function could not be effectively rebuilt after regular treatment with HAART. It is referred to as incomplete immune recovery (IIR) (2-3). Based on statistics, ~10–40% of HIV-1-infected individuals fail to achieve normalization of $CD4^+$T-cell counts despite persistent virological suppression. These patients are referred to as "inadequate immunological responders," "immunodiscordant responders," or "immunological non-responders"(2).

Patients with IIR are at higher risk of infections, cardiovascular diseases, tumors, and other diseases (4-7). However, there is no mature and effective treatment for IIR patients in clinical practice. Various interventions are being proposed, including growth hormone therapy (8), immunosuppressant therapy (9), and cytokine therapy (10). Growth hormone therapy is effective but costly. Immunosuppressants and cytokine therapy have not been clinically proven safe and effective.

Mycobacterium species, like *Mycobacterium tuberculosis*, is rich in co-antigens and therefore, a good immunomodulator with bidirectional immunomodulatory function. It can promote the transformation and proliferation of T lymphocytes,

release various lymph factors, improve the cellular immune function of patients, and enhance the resistance of the body. It can also effectively prevent the pathological damage caused by strong allergic reactions resulting from infections, such as infections caused by *Tuberculosis bacillus*. Oral *Mycobacterium vaccae* is safe, can overcome tuberculosis-associated weight loss and inflammation, reduce hepatotoxicity of tuberculosis drugs, improve sputum conversion three-fold OR 3.15, and at least cause a sixfold decrease in treatment length (11). Our previous studies have revealed that *M. vaccae* vaccine can increase the proportion of $CD4^+$T cells and $CD4^+/CD8^+$T cells in asymptomatic HIV patients, thereby improving their cellular immune function (12).

2. Objectives

This study aimed to explore a new therapeutic intervention to improve the immune recovery status of AIDS patients. *Mycobacterium vaccae* vaccine could improve the immune function of AIDS patients to some extent and facilitate immune reconstitution.

3. Methods

3.1. Study design and participants

The present exploratory research was conducted from July to December 2018. In total, 120 AIDS patients (effective cases 100) were randomly selected as research subjects from outpatient treatment at the Department of Infectious Diseases of the Ninth People's Hospital of Chongqing, Chongqing, china. The patients were aged 18-70 years old (average age: 23±2.5 years old). All of the patients signed informed consent, regardless of gender, occupation, and marital status. After three years of HAART treatment, the HIV-RNA levels of the patients were less than 103 copies/mL, and their CD4+T cell counts were less than 500/μL but more significant than 50/μL.

All patients were included and signed an informed consent form, except for AIDS patients with the following conditions: (1) those who were participating in other clinical trials at the same time or who had participated in other clinical trials within a previous month; (2) those patients whose disease was combined with uncontrolled severe opportunistic infections; (3) patients with progressive intracranial injury, combined with blood glucose abnormalities, autoimmune or hematologic diseases, early cirrhosis or malignant tumors; (4) patient who were trying to conceive or were pregnant or breastfeeding; (5) patients with abnormal liver and kidney functions; (6) patients with poor language communication or intellectual inadequacy, (7) and patients who were unable to cooperate and understand the content of treatment. All patients were randomly placed into three groups of 40 patients. Due to severe adverse reactions, changes in the treatment regimen, attrition of participants during follow-up, and other reasons, 20 patients withdrew from the study, bringing the total number of patients to 100 (35, 33, and 32 patients in groups M. vaccae vaccine, growth hormone, and placebo, respectively) (Figure 1).

3.2. Intervention

The patients were randomly divided into three groups, with each group receiving treatment for 48 weeks. Group M. vaccae vaccine received an intramuscular injection of M. vaccae vaccine (Anhui Longkema Bio-pharmaceutical Co., LTD., S20010003, 22.50 μg) once/2W for 48 weeks. The growth hormone group was subjected to an intramuscular injection of 15 IU recombinant human growth hormone (Changchun Jinsai Pharmaceutical Co., LTD., State Drug Approval S20050024, 15IU/5 mg/3 mL) per week for 48 weeks. The placebo group only received placebo (normal saline 2 mL) per week for 48 weeks.

3.3. Observational index

Flow cytometry and ELISA were used to detect the levels and changes in naive T cells, Treg cells, total CD4+T cells, T helper 1 (Th1) cells, T helper 2 (Th2) cells, and IL-7 cytokines in venous blood before treatment, after 12 weeks of treatment, after 48 weeks of treatment, and 12 weeks after the completion of treatment. Secondary infections (such as pneumocystis pneumonia, tuberculosis, and fungi) were monitored in each patient.

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3.5. Detection methods

3.5.1 CD4+T cell detection

In the first step, after collecting 2 mL of peripheral venous blood in EDTA and K2 anticoagulation vessels, CD4+T lymphocytes were counted within 24 h. In the second step, 50 μL of whole mixed blood was added to the sample tube mentioned in the first step, gently shaken, mixed well, and then incubated at room temperature for about 1 h away from light. In the third step, 50 pL of

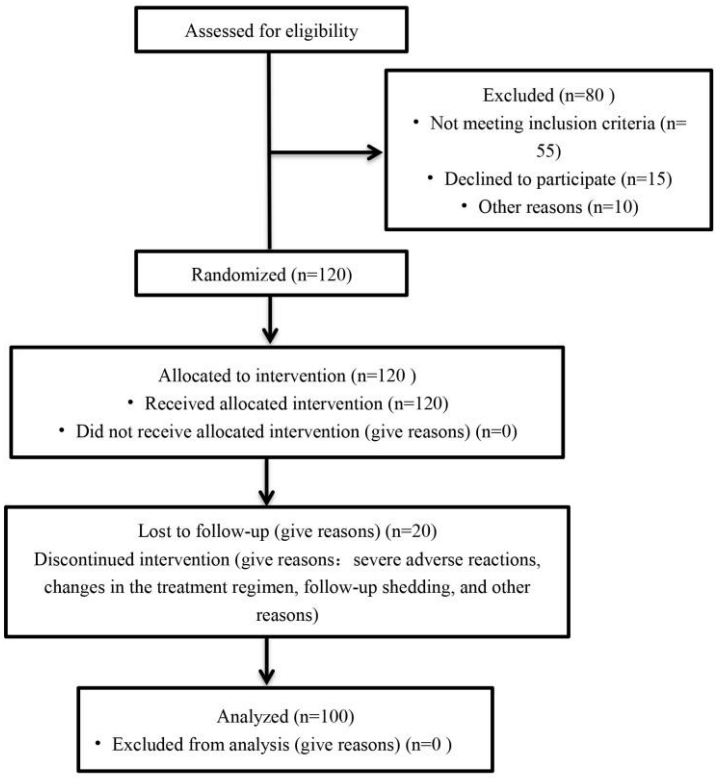

Figure 1. CONSORT - Guidelines for publishing random control

the fixative solution was added to it and placed aside for 30 min. In the fourth step, a cell analyzer (BD Company, USA) was used for detection. Flow cytometry and specific antibody fluorescence labeling measured the absolute number of CD4+T lymphocytes in the blood.

3.5.2. Treg cell detection

In total, 3 mL of venous blood for the cell isolation was obtained within 24 h at room temperature. Peripheral blood mononuclear lymphocytes were isolated using the Ficoll gradient centrifugation method, producing a cell suspension in a culture medium with a concentration of 5×106/mL. The BD FACSCalibur flow cytometer (BD Company, USA) was used to quantify Treg cell levels following the instructions of the fluorescent antibody kit of Treg cells.

3.5.3. Interleukin 7 detection

In total, 3 mL of venous blood was centrifuged for 10 min at 3,000 r/min, the supernatant was extracted and stored at -20 ℃ for later use. The enzyme-linked immunosorbent double-antibody sandwich (ELISA) method determines the serum interleukin 7 (IL-7) levels. Beller 680 enzyme linker was used, and the kits were provided by Guangzhou Weijia Technology Co., China, LTD. The relevant operations were conducted exactly as instructed by the kit manufacturer, and the IL-7 value was calculated using the standard curve drawn.

3.5.4. T helper 1 and T helper 2 detection

For this purpose, 3 mL of peripheral venous blood was collected, and BD FACSCalibur flow cytometer was used to count the number of CD4+T, Th1, and Th2 cells. The Th1 cells were CD4+T IFN-γ + positive, while the Th2 cells were CD4+T IL-4+ positive. All procedures were carried out according to the instructions of the manufacturer and operating specifications. The technician was skilled in operation, had more than three years of practical work experience, and had been assigned a professional deputy senior title for the audit duration. Flow cytometry software determined the percentage of Th1 and Th2 cells in CD4+T cells.

3.5.5 Naive T cell detection

Tracing whole blood, direct immune-fluorescence staining was used to detect the naïve T cells. The main instruments and reagents, including Facscalibur, AccuriC6 flow cytometry, mouse anti-human CD8PE, CD45RAFITC, and CD45ROAP immune fluorescent antibodies, were purchased from BD Company, USA.

3.6. Ethical Considerations

The Hospital Ethics Committee approved the research plan, and the approval number was Hospital 2018 (002).

3.7. Statistical analysis

The statistical analyses were performed in SPSS statistical software (version 23.0). Measurement

data were expressed by $\bar{x}\pm s$, chi-squared test and independent-sample t-test were used for comparison. It should also be mentioned that P < 0.05 was considered statistically significant.

4. Results

The subjects were 100 AIDS patients (68 males and 32 females) aged 18-70 years old (average age 23±2.5 years old). After 3 years of AART treatment, HIV-RNA quantification was less than 103 cepies/ml, $CD4^+$T cell count was less than 500/ul, and $CD4^+$T cell count was more than 50/ul. After a 12-week follow-up period for each patient, there were no cases of secondary infection (pneumocystis pneumonia, fungal, or tuberculosis) in the M. vaccae vaccine group, one case in the growth hormone group, and five cases in the placebo group (Table 1, 2 and 3).

Improvement of the immune function of AIDS patients by both Mycobacterium vaccae vaccine and growth hormone

After 48 weeks of treatment, it was found that thelevels of total $CD4^+$T, Th1, and naive T cells increased in AIDS patients, while the levels of IL-7 cytokines, Treg cells, and Th2 cells decreased significantly (P < 0.05) in M. vaccae vaccine and growth hormone groups. There was no rebound at 12 weeks after completion of the treatment in M. vaccae vaccine group, indicating that M. vaccae vaccine and growth hormone can improve the immune function of AIDS patients to a certain extent, as displayed in Tables 1-3 and Figure 2.

Possible higher effect of Mycobacterium vaccae vaccine on the improvement of T helper 1 index, After treatment, the total $CD4^+$T cells, Th1 cells, and naive T cells increased in the M. vaccae vaccine group, compared to the placebo group, while Treg cells, Th2 cells, and IL-7 cytokines decreased significantly (P<0.05). The only statistical difference (P<0.05) between M. vaccae vaccine and growth hormone groups was a decrease in Th1 cells in the growth hormone group, indicating that M. vaccae vaccine and growth hormone were equally effective in improving the immune function of AIDS patients (Tables 4 and 5).

Table 1. Changes of each index after treatmentin in the Mycobacterium vaccae vaccine group（35 patients）

Time	Before	12 weeks	48 weeks	50 weeks
$CD4^+$ (pcs/ul)	219.7±53.5	233.0±46.8	250.0±49.1	270.9±50.7
***P* value**		<0.001	<0.001	<0.001
Th1（%）	1.4±0.2	1.6±0.2	1.7±0.2	1.9±0.3
***P* value**		<0.001	<0.001	<0.001
Th2（%）	4.4±0.4	4.3±0.3	4.2±0.3	4.2±0.3
***P* value**		<0.001	<0.001	<0.001
Treg (%)	2.5±0.4	2.4±0.4	2.3±0.4	2.2±0.3
***P* value**		<0.001	<0.001	<0.001
Naive T cells (pcs/ul)	40.6±10.0	50.9±7.8	54.7±7.5	57.6±7.9
***P* value**		<0.001	<0.001	<0.001
IL-7（pg/ml）	3.7±0.7	3.5±0.6	3.4±0.6	3.2±0.6
***P* value**		<0.001	<0.001	<0.001

Before: before treatment; 12 weeks: after 12 weeks of treatment; 48 weeks: after 48 weeks of treatment; 50 weeks: after 50 weeks of treatment, Th: T helper, IL-7: interleukin 7. T-test, $P < 0.05$ is meaningful. The mentioned *P* value in each column is the *P* value for the column variable, compared to the variable in the previous column.

Table 2. Changes of each index after treatment in the growth hormone group（33 patients）

Time	Before	12weeks	48weeks	50weeks
$CD4^+$ (pcs/ul)	202.7±60.0	224.0±53.7	234.8±53.2	252.0±50.2
P value		<0.001	<0.001	<0.001
Th1（%）	1.5±0.2	1.6±0.2	1.7±0.2	1.7±0.2
P value		<0.001	<0.001	<0.001
Th2（%）	4.3±0.3	4.3±0.3	4.2±0.3	4.2±0.3
P value		<0.001	<0.001	<0.001
Treg (%)	2.5±0.4	2.4±0.4	2.4±0.4	2.3±0.4
P value		<0.001	<0.001	<0.001
Naive T cells (pcs/ul)	41.7±8.1	47.9±7.8	52.1±7.5	54.1±7.1
P value		<0.001	<0.001	<0.001
IL-7（pg/ml）	3.6±0.7	3.6±0.6	3.5±0.6	3.4±0.6
P value		<0.001	<0.001	<0.001

Before: before treatment; 12 weeks: after 12 weeks of treatment; 48 weeks: after 48 weeks of treatment; 50 weeks: after 50 weeks of treatment, Th: T helper, IL-7: interleukin 7. T-test, $P < 0.05$ is meaningful. The mentioned *P* value in each column is the *P* value for the column variable, compared to the variable in the previous column

Table 3. Changes of each index after treatment in the placebo group（33 patients）

Time	Before	12weeks	48weeks	50weeks
CD4⁺ (pcs/ul)	207.3±56.9	206.4±57.3	206.4±55.9	207.0±56.4
P		0.73	0.89	0.74
Th1（%）	1.7±0.2	1.6±0.2	1.7±0.2	1.6±0.2
P		0.69	0.86	0.76
Th2（%）	4.3±0.3	4.3±0.3	4.4±0.3	4.4±0.3
P		0.78	0.86	0.75
Treg (%)	2.5±0.4	2.5±0.4	2.5±0.4	2.5±0.4
P		0.69	0.71	0.58
Naive T cells (pcs/ul)	42.2±8.8	40.3±9.0	40.7±9.5	40.8±9.2
P		0.91	0.82	0.78
IL-7（pg/ml）	3.6±0.7	3.6±0.7	3.6±0.7	3.6±0.7
P		0.89	0.91	0.84

Before: before treatment; 12 weeks: after 12 weeks of treatment; 48 weeks: after 48 weeks of treatment; 50 weeks: after 50 weeks of treatment, Th: T helper, IL-7: interleukin 7. T-test, $P < 0.05$ is meaningful. The mentioned *P* value in each column is the *P* value for the column variable, compared to the variable in the previous column

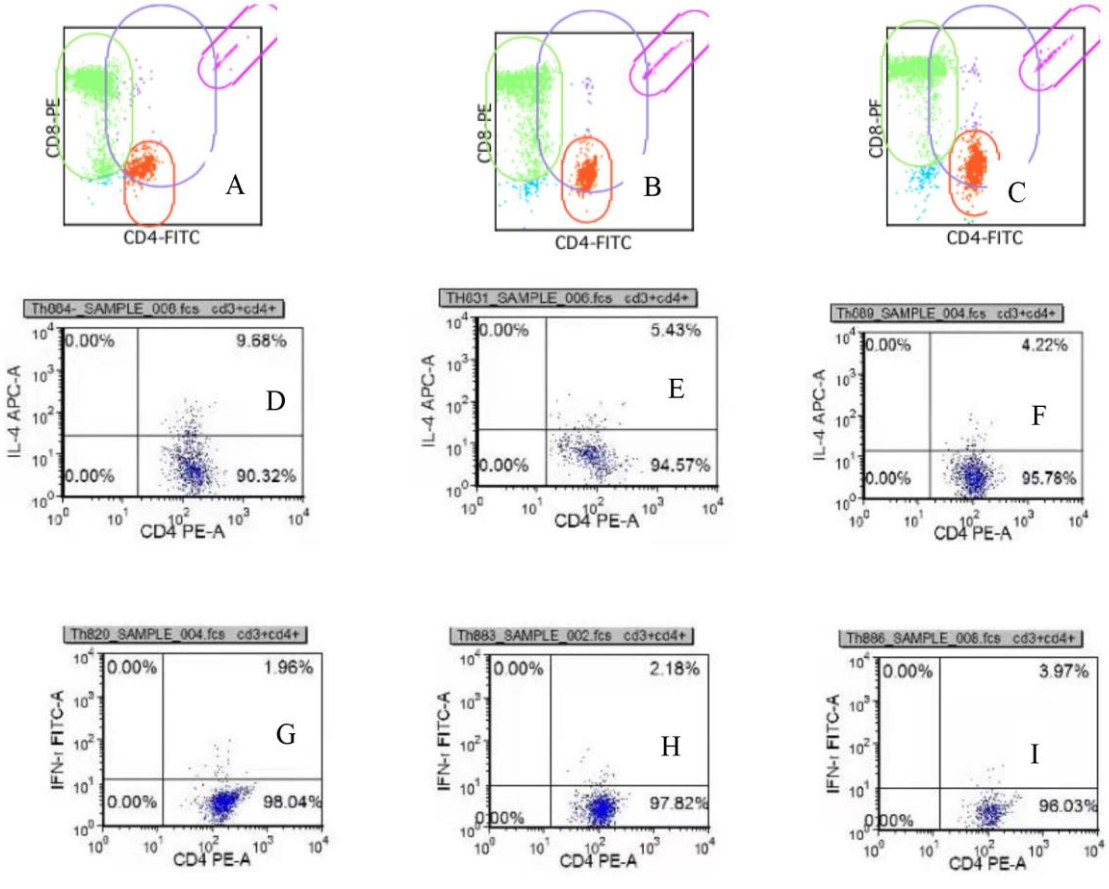

Figure 2. Flow cytometry of CD4+T cells, Th1 cells, and Th2 cells of typical cases in the *Mycobacterium vaccae* vaccine treatment group. A, B, and C represent the number of CD4+T cells examined before treatment, after 12 weeks of treatment, and after 48 weeks of treatment, respectively. D, E, and F represent the proportion of Th2 cells detected before treatment, after 12 weeks of treatment, and after 48 weeks of treatment, respectively. G, H, and I represent the proportion of Th1 cells detected before treatment, after 12 weeks of treatment, and after 48 weeks of treatment, respectively

5. Discussion

The results showed that long-term injection of the M. vaccae vaccine could increase the levels of total CD4⁺T cells, Th1 cells, and pure T cells in AIDS patients while decreasing the levels of IL-7, Treg, and Th2 cells. These changes may improve the immune reconstitution of AIDS patients.

According to relevant studies, poor immune reconstruction is related to the level of CD4⁺ T cells in AIDS patients before HAART treatment. The more reduction in CD4⁺ T cells, the more likely it leads to poor immune reconstruction (13). Findings of the present study indicated that M. vaccae vaccine may improve the immune reconstruction of AIDS patients by increasing the level of CD4⁺T cells. Even after drug withdrawal, the level of CD4⁺T cells can still increase to some extent rather than

Table 4. Effects of *Mycobacterium vaccae* vaccine and growth hormone on immune cells in AIDS patients after 50 weeks treatment

	CD4+ cells (cells/ul)	Th1 (%)	Th2 (%)
***M. vaccae* vaccine**	270.9±50.7	1.9±0.3	4.2±0.3
Growth hormone	252.0±50.2	1.7±0.2	4.2±0.3
***P* value**	0.391	0.011	0.487
***M. vaccae* vaccine**	270.9±50.7	1.9±0.3	4.2±0.3
Placebo	207.0±56.4	1.6±0.2	4.4±0.3
***P* value**	0.006	<0.001	0.003
Growth hormone	252.0±50.2	1.7±0.2	4.2±0.3
Placebo	207.0±56.4	1.6±0.2	4.4±0.3
***P* value**	0.058	0.070	0.024

Independent t-test

Table 5. Effects of *Mycobacterium vaccae* vaccine and growth hormone on immune cells in AIDS patients after 50 weeks of treatment

	Treg (%)	naïve T cells (pcs/ul)	IL-7 (pg/ml)
M. vaccae vaccine	2.2±0.3	57.6±7.9	3.4±0.6
Growth hormone	2.3±0.4	54.1±7.1	3.4±0.6
P value	0.073	0.436	0.222
M. vaccae vaccine	2.2±0.3	57.6±7.9	3.4±0.6
Placebo	2.5±0.4	40.8±9.2	3.6±0.7
P value	0.001	0.001	0.029
Growth hormone	2.3±0.4	54.1±7.1	4.2±0.3
Placebo	2.5±0.4	40.8±9.2	3.6±0.7
P value	0.109	0.009	0.332

IL-7: interleukin 7
Independent t-test

decrease. However, the exact mechanism of action is unknown.

The IL-7 can also stimulate the proliferation of mature T cells, the function of T cells, the reactivity of lymphocytokines, and the maturation of B-cell precursor cells. The IL-7 is essential for the maintenance of T cell homeostasis, and the presence of the IL-7 receptor determines its reactivity (14). Relevant studies have revealed that in HIV-infected patients, IL-7 increased while the level of IL-7 receptors decreased. However, IL-7 levels have a negative correlation with CD4+T cell levels. High IL-7 levels and low IL-7R expression decrease the number of naive CD4+T cells, resulting in IIR (15). Based on the findings of the above-mentioned studies, it was hypothesized that the M. vaccae vaccine could improve the level of CD4+T cells in AIDS patients by lowering IL-7, thereby improving poor immune reconstitution in AIDS patients.

Treg cells have immunosuppressive functions and play a role in the onset and progression of several immune diseases. Based on their immunosuppressive mechanism, these cells are classified as CD4+CD25+ Tr cells, Tr1, and Th3. The CD4+CD25+ Tr cells inhibit the immune function of activated T cells by activating CTLA-4; moreover, Tr1 secretes IL-10 to inhibit T cell proliferation and differentiation. Secretion of TGF-β by Th3 cells inhibits the proliferation and differentiation of effector cells while inhibiting cytokine production by reducing their immune function. According to different studies, patients with poorly reconstituted AIDS have a higher proportion of Treg; however, their HIV-specific immunosuppressive function is reduced. In the healthy control group, there was a strong inverse correlation between Treg cells and the presence of activated CD8+ T cells; nevertheless, no such association was found in HIV/AIDS patients. Treg cells were inversely correlated with naive CD4+T cells in patients with poor immune reconstitution but not in healthy controls and controls with good immune reconstitution (16).

Furthermore, the importance of Treg cells/naive CD4+T cells is more significant in patients with poor immune reconstitution. From this perspective, Treg cells appear to be more important in inhibiting the proliferation of innocent CD4+T cells than in inhibiting immune activation (17). Findings of the present study indicate that *M. vaccae* vaccine could reduce Treg cell level and weaken its inhibitory effect on CD4+T cell proliferation, thereby improving poor immune reconstruction in AIDS patients.

According to some studies, the better the thymus function in producing CD4+T cells, the better the recovery of CD4+T cells in patients after HAART treatment (8). A high proportion of naive T cells has been shown to reduce CD4+T cell hypoplasty (18-19). The number of naive T cells, CD31, TREC, and other parameters are commonly used in studies to represent the evaluation index of the thymus output function. Some studies have revealed that after HAART therapy, adult patients have gradually recovered the number of innocent CD4+T cells, and the output function of the thymus gland has increased rapidly and continuously, indicating that the adult thymus has an auxiliary function for its immune reconstitution (8). The high proportion of naive T cells before treatment leads to the fast recovery of its subsets, high levels of CD31 expression, and better immune reconstitution effect (20). Based on the findings, it is speculated that the

M. vaccae vaccine could improve the immune reconstitution of AIDS patients by increasing the number of naive T cells and $CD4^+$T cells.

It is now accepted that the dynamic balance of the Th1/Th2 cell ratio maintains the normal state of the body, and if the imbalance occurs, the body may shift towards the disease state. When the body is infected with HIV, it causes a decline in $CD4^+$T lymphocytes and the production of abnormal antibodies, resulting in an imbalance of cellular and humoral immunity. Related studies have found that the proportion of Th2 cytokines in AIDS patients is higher, implying that the development of AIDS may be linked to the transformation of the immune response of the body from Th1 to Th2 (21). These findings indicate that the *M. vaccae* vaccine might increase the proportion of Th1 cells while decreasing the proportion of Th2 cells in AIDS patients, allowing Th1/Th2 to develop towards a normal homeostasis trend, thereby regulating the balance of Th1/Th2 cells in AIDS patients and improving their immune reconstitution. The specific mechanism of down-regulation of Th2 cells and up-regulation of Th1 is unknown.

At present, there are no studies on the use of cow mycobacterium vaccine to improve immune reconstitution in AIDS patients, and this study is proposed for the first time. The sample size of this study was relatively small, and the follow-up time in the later stage was insufficient. It is suggested that further studies be performed with more samples and extended time to be more indicative of the problem.

6. Conclusion

In conclusion, *M. vaccae* vaccine can improve the reconstructive immune dysfunction of AIDS patients to some extent and its efficacy is comparable to that of growth hormone at a lower price. Therefore, it provides a new treatment method for improving the immune reconstitution function of AIDS patients. It is worthwhile to further investigate the specific mechanisms and conventional treatment methods to improve immune reconstitution.

Acknowledgments

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Footnotes

Conflicts of Interest: The authors declare that they have no conflict of interest.

Author Contribution: Mao-Rui He, Wan-Shu Zeng designed this project; Wan-Shu Zeng, Lu-Lu Jia, Xue-Ou Wen, Hong Yang carried out experiments, and collected data; Bing Liao tested data, Wan-Shu Zeng analyze data, Wan-Shu Zeng and Mao-Rui He wrote paper.

Informed Consent: All subjects signed informed consent.

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Ethical statements: The research plan was had been approved by the Hospital Ethics Committee, and the approval number of the ethics review approval was Hospital 2018 (002).

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