# Original Article

# Immune Function Alterations during 12 Weeks of Abstinence in Heroin Users

(heroin user / abstinence / immune)

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**Abstract. The intent of the study was to evaluate immune system changes during 12 weeks of abstinence in heroin users. We recruited men (N = 65) aged 18–45 years and collected demographic and heroin use pattern data. Serum blood levels of total interleukin 2 (IL-2), interferon γ (IFN-γ), immunoglobulin (Ig) A, IgG, and IgM were assessed at five time points. The IL-2 level was increased on day 84 as compared to that in healthy controls. The IFN-γ level was higher in heroin users than in healthy controls between days 0 and 28, and was decreased on day 84. IgG and IgM levels in heroin users were higher than those in healthy controls in our 12-week study, and were in positive correlation with the way of using the drug, duration of heroin dependence, and daily heroin intake. Our data revealed that the immune system was not restored during the 12 weeks of heroin withdrawal.**

## Introduction

Heroin abuse is one of the major public health problems and a chronic medical condition. Chronic administration of heroin produces several immunosuppressive effects, by directly activating the opioid receptors present on lymphocytes and macrophages (Stefano et al., 1996; Nelson et al., 2000) or by indirectly acting on the central nervous system (CNS) (Peterson et al., 1993, 1998; Fecho et al., 1996; McCarthy et al., 2001). As a result, infectious diseases such as HIV, hepatitis B and C, endocarditis, and other opportunistic infections are common among heroin abusers (Doherty et al., 2000; Tennant, 2001).

It has been reported that decreased natural killer cell activity, lymphocyte proliferation, nitric oxide production, and immune cell recruitment occur in heroin-treated experimental animals or in heroin abusers (Eisenstein and Hilburger, 1998; Pacifici et al., 2000; Martin et al., 2010). Weber demonstrated that chronic heroin administration did not alter the total number of leukocytes per spleen, whereas cessation of heroin administration resulted in a significant decrease in rats (Weber et al., 2004).

Long-term changes in immune function have not been fully reported after acute or protracted withdrawal, particularly during the gradual relief of protracted withdrawal. As we know, without drug or medical support, symptoms of heroin withdrawal appear 8–12 h after the last dose. At 7–10 days after the last dose, gross symptoms disappear (Leguit et al., 1982). The present study evaluated whether the immune system of abstinent patients returned to normal values during a consecutive 12-week period. Lymphocytes are important components and central mediators of the immune response. Their function is regulated by cytokines, which can be affected by drug abuse and stress. We measured production of the T helper1 (Th1) cytokines, interleukin 2 (IL-2) and interferon γ (IFN-γ), which link cellular immune responses with tissue injury (Romagnani, 1994; Mosman and Sad, 1996). The production of antibodies that contribute to the humoral immune system, including immunoglobulin (Ig) A, IgG, and IgM, was also evaluated (Pier et al., 2004). The balance between cellular and humoral immunity is needed to maintain the immune system homeostasis (Charlton and Lafferty, 1994).

We hypothesized that 12-week abstinence from heroin induces normalization of immune parameters in the patients.

## Material and Methods

### *Patients and ethics*

This study was carried out at the Compulsory Detoxification Centre of Sichuan Province in China. Sixty-five male patients with heroin addiction were recruited

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Abbreviations: CNS – central nervous system, IFN-γ – interferon γ, Ig – immunoglobulin, IL-2 – interleukin 2, TNF-α – tumour necrosis factor α.

between March 2009 and June 2010 in a controlled setting, meeting the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, standards. Participants remained free from opioids for at least 12 weeks without pharmacological heroin detoxification.

The inclusion criteria included patients aged 18 to 45 years with a diagnosis of heroin dependence, a heroin-positive urine analysis detected by thin-layer chromatography, final drug in the last 8 to 36 h, and written informed consent. The exclusion criteria included serious opiate withdrawal symptoms including disturbance of consciousness, serious violence, or severe dehydration; attempted suicide, current psychiatric illnesses, or dementia; serious infectious diseases, serious disease of the heart, lung, kidney, liver, central nervous system, or haematopoietic cancer; an inability to understand the consent process. Twenty-nine age-matched male healthy controls who had never used illicit psychotropic drugs or misused alcohol were recruited from the local community. All participants were screened with the Structured Clinical Interview for DSM-IV disorder (SCID), physical examination, urine drug screens, and electrocardiogram.

This study was approved by the Medical Ethics Committee of West China Hospital of Sichuan University, and the trial conformed to the principles of the Declaration of Helsinki. All patients signed informed consent forms and were able to withdraw from the study at any time.

### *Determination*

Blood samples from 65 patients were collected at baseline (day 0, D0) and D10, D28, D56, and D84 at about the same time (9 AM). Blood samples were kept in non-heparinized tubes immediately after being drawn, allowed to clot at 37 °C for 5 min, and centrifuged at 2500 rpm for 15 min (L600A, Cence, China). The serum was separated and stored at −70 °C until tests were conducted at West China Hospital. IL-2 and IFN-γ levels were determined by enzyme-linked immunosorbent assay by means of commercially available kits for human cytokines (Bender MedSystems, Vienna, Austria). Sensitivity of the method for IL-2 and IFN-γ evaluation was 9.1 pg/ml and 0.99 pg/ml. IgA, IgM, and IgG levels were determined by rate nephelometric assay (IMMAGE-800, Beckman Coulter, Brea, CA). Sensitivity of the method was 67 mg/l, 42 mg/l, and 0.333 g/l, respectively. Only one blood draw was made for each control subject.

Tests were performed by a technician blinded to the sample identities.

*Table 1. Characteristics of patients with heroin dependence*

| **Demographic data** | **Heroin users** |
|---|---|
| Duration of heroin dependence (months) | 67.68 ± 63.55 |
| Daily heroin intake (g) during the past week | 0.48 ± 0.54 |
| Way of using the drug (cigarettes/injection/other) | 37/28/0 |
| Time from the last use (hours) | 19.12 ± 5.56 |
| Amount of the last use (g) | 0.16 ± 0.21 |

Data are shown as the number or the mean ± SD.

*Table 2. Correlation between the levels of IFN-γ, IL-2, IgA, IgG, IgM, the way of using the drug, duration of heroin dependence, and daily heroin intake in the heroin users on D0*

| | **Way of using the drug** | | **Duration of heroin dependence** | | **Daily heroin intake** | |
|---|---|---|---|---|---|---|
| | **Pearson correlation** | **P value** | **Pearson correlation** | **P value** | **Pearson correlation** | **P value** |
| IFN-γ | -0.105 | 0.253 | -0.237 | 0.007 | 0.092 | 0.305 |
| IL-2 | 0.122 | 0.186 | -0.127 | 0.153 | 0.005 | 0.952 |
| IgA | 0.138 | 0.133 | 0.113 | 0.205 | 0.033 | 0.714 |
| IgG | 0.328 | < 0.001 | 0.187 | 0.035 | 0.186 | 0.036 |
| IgM | 0.373 | < 0.001 | 0.213 | 0.016 | 0.214 | 0.016 |

*Table 3. Correlation between the levels of IFN-γ, IL-2, IgA, IgG, IgM, way of using the drug, duration of heroin dependence, and daily heroin intake in the heroin users on D84*

| | **Way of using the drug** | | **Duration of heroin dependence** | | **Daily heroin intake** | |
|---|---|---|---|---|---|---|
| | **Pearson correlation** | **P value** | **Pearson correlation** | **P value** | **Pearson correlation** | **P value** |
| IFN-γ | 0.062 | 0.629 | -0.121 | 0.324 | -0.003 | 0.978 |
| IL-2 | 0.102 | 0.423 | -0.019 | 0.880 | 0.213 | 0.082 |
| IgA | 0.001 | 0.992 | 0.282 | 0.020 | -0.028 | 0.819 |
| IgG | 0.308 | 0.013 | 0.041 | 0.739 | 0.196 | 0.110 |
| IgM | 0.171 | 0.177 | 0.205 | 0.093 | 0.159 | 0.195 |

### *Statistical analysis*

All data were documented with Epidata 3.0 by two independent individuals and were secured after checking. All data were analysed with SPSS version 19.0. The patient characteristics data, including age, duration of heroin dependence, time from the last drug use, amount of the last drug use, and average daily amount of use for the past week at baseline, were expressed as the mean ± SD. The Student's *t*-test was used for age. Repeated-measures analysis of variance was performed to evaluate significant differences in immune markers. The correlation among the different parameters in the patient group was evaluated by means of the Pearson correlation test. Statistical significance was preset at 0.05 using a 2-tailed test.

## Results

Patient characteristics are shown in Table 1, including duration of heroin dependence, method of drug use, time from the last use, amount of the last use, and average daily amount used during the past week. Statistical analyses between heroin users and healthy controls showed that they were comparable in age (heroin users: 28.82 ± 4.05 and healthy controls: 28.10 ± 5.29, P = 0.477).

On D0, D10, D28, and D56, the IL-2 levels in heroin users were consistently lower than in healthy controls (heroin users: 5.82 ± 2.81 pg/ml on D0, 4.74 ± 2.35 pg/ml on D10, 3.87 ± 1.77 pg/ml on D28, 5.82 ± 3.17 pg/ml on D56; healthy controls: 9.45 ± 5.28 pg/ml, P < 0.001). However, on D84, the IL-2 levels in heroin users (19.51 ± 6.72 pg/ml) were higher than in the controls, displaying a four-fold increase over four weeks (P < 0.001, Fig. 1A).

IFN-γ levels were higher in heroin users than in healthy controls from D0 to D28 (D0: P < 0.001; D10: P = 0.031; D28: P = 0.002; healthy controls: 4.31 ± 4.93 pg/ml). The IFN-γ levels gradually decreased between D56 (3.59 ± 2.50 pg/ml, P = 0.350) and D84 (1.42 ± 0.77, P < 0.001, Fig. 1B).

The IgA level did not differ between heroin users and healthy controls from D0 to D84 (P = 0.183 at D0; P = 0.129 at D10; P = 0.068 at D28; P = 0.178 at D56; P = 0.098 at D84). The normal IgA level was 2.31 ± 1.88 g/l (Fig. 2A).

IgG and IgM levels in heroin users were significantly higher than in healthy controls during our 12-week study

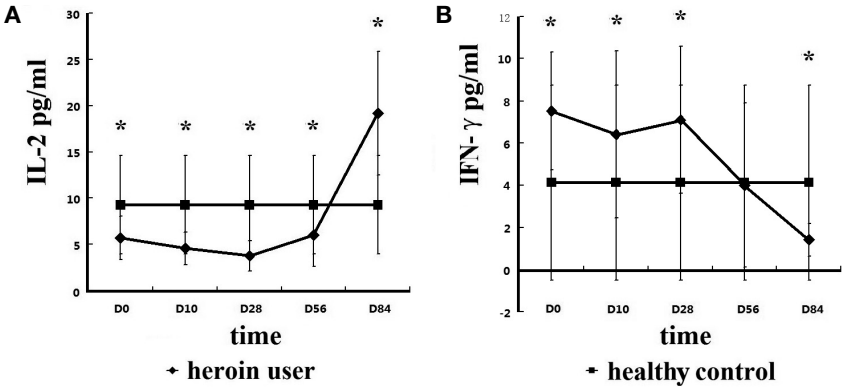


*Fig. 1.* Changes of immunological factors in heroin users and healthy controls. IL-2 concentration (**A**), IFN-γ concentration (**B**) changes during 12-week observation. Data are presented as mean ± SEM concentrations for each time point. *P < 0.05, healthy controls (N = 29) and heroin users (N = 65).

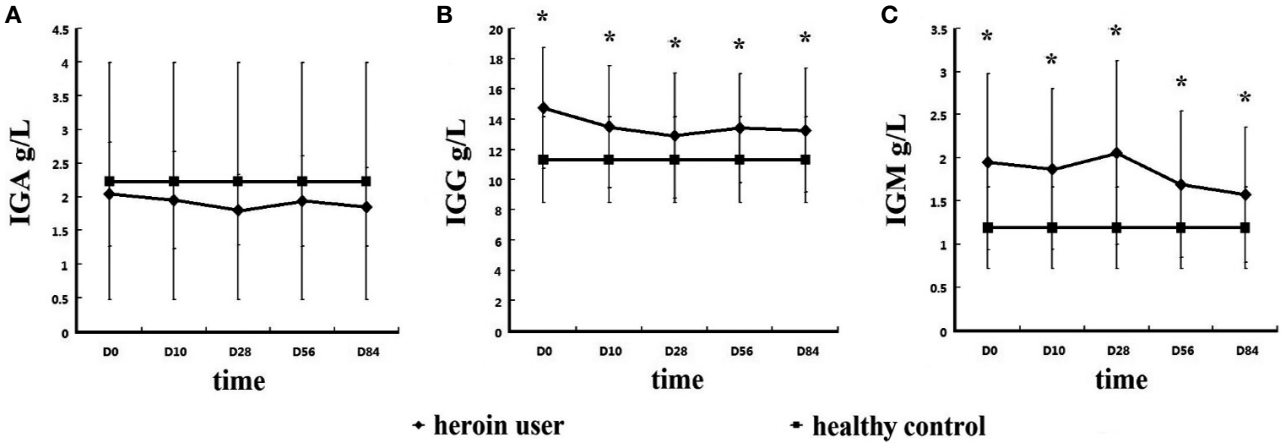


*Fig. 2.* Changes of immunological factors in heroin users and healthy controls. IgA concentration (**A**), IgG concentration (**B**) and IgM concentration (**C**) changes during 12-week observation. Data are presented as mean ± SEM concentrations for each time point. *P < 0.05, healthy controls (N = 29) and heroin users (N = 65).

(IgG: healthy controls: 11.47 ± 3.01 g/l, $P < 0.001$ on D0, $P = 0.007$ on D10, $P = 0.016$ on D28, $P = 0.019$ on D56, $P = 0.041$ on D84; IgM: healthy controls: 1.14 ± 0.42 g/l, $P < 0.001$ on D0, D10, and D28, $P = 0.001$ on D56, $P = 0.009$ on D84, Fig. 2B, C).

In heroin users, significant positive correlations were observed between the way of using the drug and the IgG level (0.328, $P < 0.001$), between the duration of heroin dependence and the IgG level (0.187, $P = 0.035$), between the daily heroin intake and the IgG level (0.186, $P = 0.036$), between the way of using the drug and the IgM level (0.373, $P < 0.001$), between the duration of heroin dependence and the IgM level (0.213, $P = 0.016$), between the daily heroin intake and the IgM level (0.214, $P = 0.016$) on D0, and between the duration of heroin dependence and the IgA level (0.282 $P = 0.020$), between the way of using the drug and the IgG level (0.308, $P = 0.013$) on D84. A significant negative correlation was observed between the duration of heroin dependence and the IFN-γ level (-0.237, $P = 0.007$; Table 2, Table 3) on D0.

## Discussion

In this 12-week study, the IL-2 level significantly increased between D56 and D84, whereas the IFN-γ level significantly decreased between D56 and D84. The IgG and IgM levels were significantly higher in the heroin users than those in the healthy controls during the monitored 12 weeks. In addition, the IgG and IgM levels positively correlated with the way of using the drug, the duration of heroin dependence, and the daily heroin intake, indicating that injection drug use, long-lasting heroin use and increased heroin intake may be in direct proportional relationship with the IgG and IgM levels. Studies have reported increased levels of IL-2, IFN-γ, IgG and IgM in active (non-abstinent) heroin users (Zajícová et al., 2004; Simonovska et al., 2011). However, one study has reported decreased levels of IL-2 and IFN-γ in heroin withdrawal subjects (Zaki et al., 2006). Weber reported that chronic heroin produced a significant increase in tumour necrosis factor α (TNF-α) and nitric oxide production, and 10 days following stop of heroin administration, TNF-α and nitric oxide production returned to normal levels in rats (Weber et al., 2004). We investigated immune function changes among patients undergoing heroin withdrawal. We did not find peripheral cytokine levels and antibodies returned to the normal in the present study.

Chronic opioid use can cause neuronal degeneration and neuronal damage (Chan et al., 2015). Cytokines exert both positive and negative impacts on the central nervous system (CNS) regeneration. The inhibitory immune factors are up-regulated around the CNS lesion site, so that CNS regeneration is difficult (Dooley et al., 2014). IFN-γ acts as an inhibitory inflammatory cytokine in the course of neurological diseases such as cerebral trauma (Schmitz and Chew, 2008), stroke (Liesz et al., 2009) and multiple sclerosis (Lees et al., 2008). Dooley et al. (2014) thought that a high level of IFN-γ would damage neurogenesis and stem cells. However, IL-2 might exert a positive impact on the process of CNS regeneration in the rats (Sarder et al., 1996; Wang et al., 2001). In the present study, the decreased IFN-γ and increased IL-2 levels might be associated with the benefits of CNS regeneration after D56. In addition, studies suggest that immune activation is enhanced in response to stress. For example, IL-2 production responses to the hypothalamic-pituitary-adrenal axis were higher during an examination period than during a non-examined period (Kang et al., 1996; Bosch et al, 1998; Koh, 2001; Koh et al., 2006). Others studies indicate that stress down-regulates immune functions (Glaser et al., 1986, 1987, 1990, 1991; Marchesi et al., 1989; Dobbin et al., 1991; Fawzy, 1995; Deinzer and Schuller, 1998; Marucha et al., 1998; Rojas et al., 2002). This activated immune response may be associated with the intensity and/or duration of stress, and may be enhanced when the stress is mild to moderate in intensity (Weiss and Sundar, 1992).

Reportedly, IgM and IgG antibodies were elevated in heroin users, which gave a possible explanation of some somatic changes in heroin users, such as kidney complications, autoimmune thrombocytopenia, arterial or venous thrombosis (Savona et al., 1985; Crowe et al., 2000; Nikolova et al., 2002). The studies reported that the antibody level correlated with the duration of heroin dependence and injection heroin use (Nikolova et al., 2002). Our results have confirmed this positive correlation with injection drug use, long-lasting heroin use and increased heroin intake for the higher IgG and IgM levels in acute heroin withdrawal.

In summary, our study reveals that abstinent heroin users displayed alterations in immune cytokines and antibodies during 12 weeks of heroin withdrawal, which did not return to the normal in the present study. Future long-term studies should evaluate the gradual reversibility of immunological parameters to normal levels in the abstinent heroin users.

## Limitations

Abstinent participants in a controlled setting may differ from those in a free-living environment, which could affect biological and immune system changes after acute abstinence. In addition, more biological indices and female subjects should be taken into account in a larger homogeneous sample of heroin-dependent patients in future studies.

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