Development of a Biomarker System for Detecting Exposure to Waterborne Viral Pathogens

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ABSTRACT

In the present study, interferon gamma (IFN-γ) was selected as a biomarker in an animal model for a viral exposure study. To determine whether IFN-γ can be used as a biomarker for the viral infection, twelve-week-old BALB/c mice were intraperitoneally injected with coxsackievirus B3 or B4 diluted in phosphate-buffered saline (PBS). Control group mice were injected with PBS only. Four months after viral infection, mouse spleen and thymus were collected. T lymphocytes were isolated from the organs and assayed for the release of IFN-γ after *ex vivo* stimulation (incubation) with viral antigens, phytohaemagglutinin (PHA) and PBS, respectively. The level of IFN-γ released by T lymphocytes was examined by antibody-capture enzyme-linked immunosorbent assay (ELISA). Our results demonstrated that IFN-γ produced by memory T cells is virus specific and can be used as a biomarker in viral exposure studies. The results of this study indicate that the measurement of IFN-γ may provide an ideal biomarker for human exposure studies related to waterborne microbial pathogens. Furthermore, this new approach may offer a better and more accurate method for risk/exposure assessment to microbial pathogens.

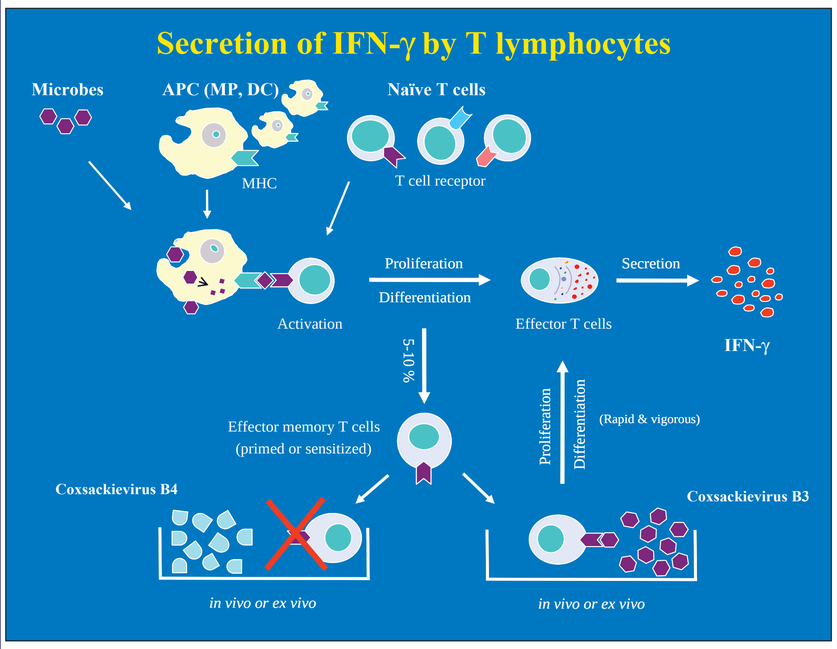
INTRODUCTION

EPA has published a drinking water Contaminant Candidate List (CCL) that includes waterborne pathogens and chemicals that may be considered for regulation at a future date. For each contaminant on the CCL, the Agency will need sufficient data to conduct analyses on the extent of exposure and the risk posed to populations via drinking water. Previous studies indicated that exposure to some microbes, including the CCL pathogen coxsackievirus B3 and B4, may be associated with serious long-term health consequences (sequela), such as myocarditis and type-1 diabetes. However, little is known about waterborne-associated infections by these microbes and their linkage to chronic diseases. In addition, surveillance reports by EPA and the Centers for Disease Control and Prevention (CDC) suggest that etiological agents cannot be identified in a large percentage of outbreaks. It is suspected that many of the unidentified pathogens may be viruses. Our inability to culture many viral pathogens has made their identification very difficult. Therefore, it is important to develop biomarkers of viral exposure to be able to investigate the relationship between the environmental exposure and human diseases.

OBJECTIVE OF STUDY

The objective of this study was to develop a biomarker system for detecting exposure to waterborne pathogens

PRINCIPLE & RATIONALE OF THIS STUDY



MATERIALS AND METHODS

Mice Twelve-week-old female BALB/c mice were purchased from the Charles River Laboratories International Inc. (Wilmington, MA) and used in this study.

Viruses Coxsackievirus B3 (CVB3), Nancy strain and Coxsackievirus B4 (CVB4), J.V.B. strain were obtained from the American Type Culture Collection (Manassas, VA).

Antibodies Rat anti-mouse IFN-γ monoclonal antibody (mAb) was purchased from R&D Systems Inc. (Minneapolis, MN). Biotin-labeled rat anti-mouse IFN-γ mAb was obtained from Pierce Biotechnology Inc. (Rockford, IL).

Inoculation of mice

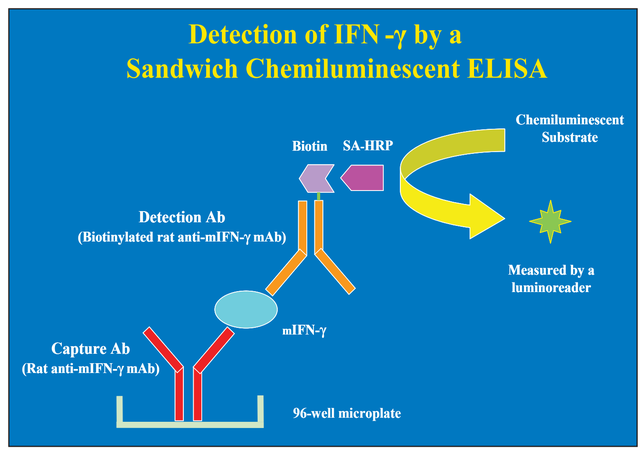
Mice were intraperitoneally injected with 0.2 ml of 10^4 PFU/ml of either CVB3 or CVB4 diluted in sterile phosphate-buffered saline (PBS). Negative control mice were injected only with PBS. All injected mice were housed in microisolator cages, given sterile water and monitored daily. All animal procedures were approved by the Institutional Animal Care and Use Committee of National Exposure Research Laboratory of U.S. EPA.

Stimulation of T lymphocytes and cell culture

Four months after inoculation mice were sacrificed, and spleen and thymus were harvested. Spleen and thymus cells were isolated from the organs and washed 3 times with RPMI 1640. The cells were evenly distributed to 24-well plate and stimulated with CVB3, CVB4, PBS and phytohaemagglutinin (PHA), respectively. The cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 IU/ml of penicillin and 100 µg/ml of streptomycin for 3 days at 37ºC in a humidified atmosphere with 5% CO_2.

Detection of IFN-γ in cell culture supernatant

Three days after stimulation of T cells the levels of IFN-γ present in the supernatants of T cell cultures were determined by enzyme-linked immunosorbent assay (ELISA). In order to increase sensitivity of the assay, biotinylated detection antibody and chemiluminescent substrate were used. ELISA results were measured by a multi-detection microplate reader (Synergy™ HT, Bio-Tek Instruments, INC., Winooski, Vermont).



RESULTS

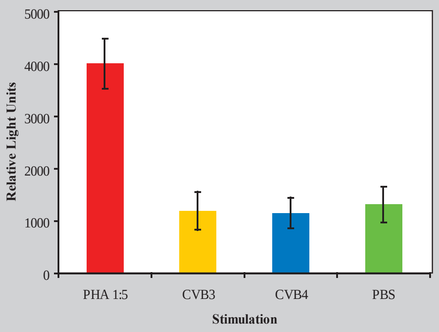


Figure 1. No detectable release of IFN-γ by virus stimulated T cells from PBS inoculated mice

Three days after stimulation of T cells with CVB3 and CVB4, levels of IFN-γ from both cell culture supernatants were not increased compared with that of negative control stimulated with sterile PBS buffer. These negative results indicated that virus-specific memory T cells were not developed from these control mice unless they were previously infected by the viruses.

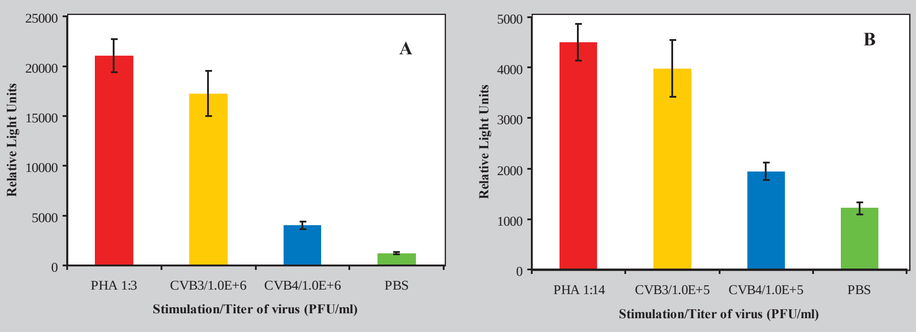


Figure 2. Release of IFN-γ by CVB3 specific memory T cells from CVB3 infected mice

In order to obtain virus educated or sensitized (memory) T cells, mice were injected with CVB3. Four months after infection, T cells were isolated from mouse spleen and thymus and then stimulated with both CVB3 and CVB4. Figure 2 (A) shows that 3 days after stimulation of T cells, the level of IFN-γ was markedly increased from the cell culture stimulated with CVB3 compared with that stimulated with CVB4. This result indicated that CVB3 memory T cells recognized T cell epitope from CVB3, not from CVB4. Figure 2 (B) shows a same stimulation pattern as one shown in figure 2 (A) when the cell cultures were stimulated with virus dose that was ten fold lower than the previous one.

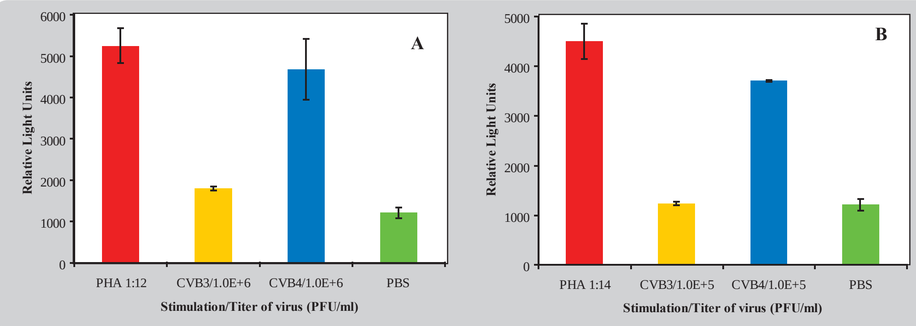


Figure 3. Release of IFN-γ by CVB4 specific memory T cells from CVB4 infected mice

Figure 3 shows exactly opposite result compared with figure 2. In this experiment mice were previously infected by CVB4. Therefore, immune system of these mice developed CVB4 educated T cells. When the memory T cells isolated from these mice were stimulated with two different doses of CVB4, the levels of IFN-γ were significantly increased compared with ones stimulated with CVB3.

CONCLUSION

The IFN-γ produced by memory T cells is virus-specific and can be utilized as a biomarker for detecting exposure to waterborne viral pathogens

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