Journal of Clinical Diabetes

Colfer, J Clin Diabetes 2023, 7:5

Mini Review Open Access

The Spread of *Chlamydia pneumoniae* from the Lungs to the Peripheral Blood is Facilitated by the Development of Diabetes in Non-Diabetic Diabetic Mice

Dan Colfer*

Department of Prediabetes, University of the Philippines, Quezon, Philippines

Abstract

We investigated the possibility of a link between the spread of *Chlamydia pneumoniae* from the lungs to the peripheral blood and the development of diabetes in NOD mice that were not obese diabetics. After multiple intranasal inoculations, we detected bacteria in the lung of NOD mice with diabetes and institute of cancer research, USA mice using real time Reverse Transcription Polymerase Chain Reaction (RT-PCR) with *C. pneumoniae* 16S rRNA primers. However, the prevalence of bacteria in NOD mice without diabetes, pre-diabetic NOD mice and non-diabetic retired NOD mice was very low. The bacteria were only found in hydrocortisone cultured mononuclear cells from the diabetic NOD mice's peripheral blood. The PBMCs were determined to be positive by RT-PCR and immunostaining with fluorescein isothiocyanate conjugated ant *Chlamydia* monoclonal antibody also revealed the presence of bacterial antigens in the lungs. However, the cultivation method and inclusion forming units assay failed to detect *C. pneumoniae* in the PBMCs of all NOD mice. Additionally, it was confirmed that *C. pneumoniae* intranasal inoculation had no effect on the development of diabetes in NOD mice.

Keywords: *Chlamydia pneumoniae*; Diabetes; Dissemination; Inclusion forming units; Non-obese diabetic mice; Peripheral blood mononuclear cells; Real time reverse transcription-polymerase chain reaction

Introduction

The likelihood that the DNA or antigen of *C. pneumoniae* is involved in the pathogenesis of atherosclerosis has increased following the discovery of it in coronary atherosclerotic plaque and peripheral blood of cardiovascular patients [1]. In addition, a number of studies indicate that both culture and real time Reverse Transcription Polymerase Chain Reaction (RT-PCR methods can detect viable *C. pneumoniae* in atherosclerotic plaques from elderly patients with coronary heart disease [2]. In addition, our most recent research demonstrated that healthy donors' Peripheral Blood Mononuclear Cells (PBMCs contained viable *C. pneumoniae*, suggesting that the presence of viable bacteria in the blood stream may be a risk factor for atherosclerosis [3]. Regarding this, systemic dissemination of *C. pneumoniae* from the lung to the spleen, heart or aorta in animal models has already been reported in several studies investigating the pathogenic role of *C. pneumoniae* in atherosclerosis [4]. Therefore, rather than components derived from dead bacteria stimulating the atherosclerotic process, it is possible that the presence of *C. pneumoniae* in blood vessels is directly associated with the formation of atherosclerotic plaque. However, the mechanisms by which *C. pneumoniae* spreads from the lungs to the peripheral blood are still poorly understood. Diabetes is also a risk factor for atherosclerosis, which is widely acknowledged [5]. Albeit the job of *C. pneumoniae* disease in diabetic patients is as yet not satisfactory, the improvement of atherosclerotic plaques in diabetic patients might be advanced quickly by the presence of *C. pneumoniae* in blood. In addition, the development of diabetes is likely to result in immune suppression, resulting in compromised host permitting exceptional infection [6,7]. This escapable condition from the host's defences may allow *C. pneumoniae* to survive and grow for an extended period of time in either lung or peripheral blood cells. As a result, the development of diabetes may contribute to the spread of C. pneumoniae throughout the body. Although it is known that Non-Obese Diabetic (NOD) mice spontaneously develop autoimmune diabetes, which shares many characteristics with human diseases, there have never been any animal studies to evaluate this hypothesis.

Therefore, we investigated the susceptibility of NOD mice to *C. pneumoniae* systemic dissemination to confirm a possible association between the developments of diabetes in NOD mice [8-10].

Literature Review

The extraction of total RNA and the examination of *C. pneumoniae* 16S rRNA transcripts

As previously mentioned, total RNA extraction, cDNA synthesis and real time RT-PCR with *C. pneumoniae* 16S rRNA primers were carried out. RNeasy Mini Kit Qiagen was used to extract total RNA from the treated PBMCs in accordance with the manufacturer's instructions for bacterial cells. DNase Ambion, Austin, Texas, United States, was used to treat total RNAs. Real time PCR, which did not use reverse transcription, confirmed that the RNAs had been stripped of their DNA. In a 20 l commercial reaction mixture, Avian myeloblastosis virus reverse transcriptase and random primers were used to perform RT on 1 g of RNA; system for reverse transcription; Madison, WI, USA: Promega. Real time RT-PCR analysis of each target sample for *G3PDH* gene mRNA expression also confirmed the

***Corresponding author:** Dan Colfer, Department of Prediabetes, University of the Philippines, Quezon, Philippines; E-mail: colfer_d3@gmail.com

Received: 16-March-2023, Manuscript No. JCDS-23-91912; **Editor assigned:** 20-March-2023, PreQC No. JCDS-23-91912 (PQ); **Reviewed:** 03-April-2023, QC No. JCDS-23-91912; **Revised:** 05-June-2023, Manuscript No. JCDS-23-91912 (R); **Published:** 12-June-2023, DOI: 10.4172/jcds.1000193

Citation: Colfer D (2023) The Spread of Chlamydia pneumoniae from the Lungs to the Peripheral Blood is Facilitated by the Development of Diabetes in Non-Diabetic Diabetic Mice. J Clin Diabetes 7: 193.

reproducibility of the amount of intact RNA input and the effectiveness of cDNA synthesis. The master mixture QuantiTect SYBR green PCR kit was used to perform real time PCR on the 2 l of cDNA that were produced; Qiagen with a primer for the 16S rRNA sense of *C. pneumoniae*, 5′-GGA CCT TAG CTG GAC TTG ACA TGT-3′; antisense, 5′-CCA TGC AGC ACC TGT GTA TCT G-3′, in a gene Amp 5700 sequence detection system manufactured by PE bio systems in Foster city, California, USA. The aftereffects of Impact search showed that the preliminaries utilized for on-going RT-PCR were explicit for *C. pneumoniae* identification information not shown. The thermal cycling conditions were 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds, 60°C for 1 minute and 72°C for 20 seconds. The specificity of the PCR products was confirmed by looking at the melting Temperature profile (Tm) for each PCR run. Just examples with a Tm=76.9°C were distinguished as sure. Direct oligonucleotide sequencing of the PCR products from Sawady, Tokyo, Japan, also confirmed the presence of amplified target genes in a positive specimen. Standards were HEp-2 cells infected with *C. pneumoniae* that had their DNA serially diluted. The cDNA duplicate number enhanced by still up in the air from the standard bends. As a negative control for PCR, the RT product made from uninfected HEp-2 cells was also used. At least three PCR tests were performed on each of the cDNA samples. One copy is the theoretical limit of PCR's sensitivity. However, the lower detection limit for real time PCR was 0.1 copies per PCR due to the possibility that the number of IFUs represents more bacterial particles than infectious bacteria. In order to avoid contamination from previous steps, an aerosol resistant tip was utilized. To prevent contamination, all laboratory procedures were carried out in a safety hood with filtered airflow. The various rooms were used for the total RNA extraction, PCR mixture preparation and PCR run.

Discussion

The detection of *Pneumoniae* in human Peripheral Blood Mononuclear Cells (PBMCs) is more sensitive than the demonstration of a bacterial genome. Regarding this, a number of studies have reported persistent *C. pneumoniae* that occasionally occurs *in vivo*. This stage has been described as a type of *Chlamydia* host cell interaction known as chronic infection in which the bacteria are non-infectious and do not divide. However, the IFU assay, a conventional culture based method, is unable to detect persistent bacteria. As a result, immunostaining with MoAb was used as the best method of detection in the current study, which relied on the detection of bacterial RNA. *C. pneumoniae* was frequently detected by RT-PCR in cultured PBMCs and lung tissues of diabetic NOD mice, indicating the spread of cells containing viable bacteria into the bloodstream. The aftereffects of immunostaining of lung tissues and refined PBMCs with chlamydial immunizer additionally support that *C. pneumoniae* are available in lung tissues and platelets, since lung tissues and refined PBMCs uncovered chlamydial explicit consideration bodies by staining with explicit immunizer. When lung inflammation subsided, cultured PBMCs from diabetic NOD mice could still successfully detect bacteria. As a result, it's possible that the presence of bacteria in PBMCs has nothing to do with any inflammation in the lungs, allowing for the non-specific transfer of pathogens to systemic tissues. In addition, the development of diabetes is likely to be one of the events that promote the dissemination through a consequence of productive lung infection due to the fact that dissemination of bacteria failed to occur in either non-diabetic NOD mice or ICR mice that have a genetic background that is comparable to that of NOD mice. It's interesting to note that NOD mice without diabetes were extremely resistant to *C. pneumoniae* lung infection. For NOD mice to develop diabetes, the activation of insulitic cell auto reactive T cells in the pancreatic islets is essential.

Conclusion

Therefore, non-diabetic NOD mice's strong resistance against *C. pneumoniae* lung survival and growth may be influenced by this ingoing activation of auto reactive T cells before diabetic development.

References

1. Altannavch T, Roubalova K, Broz J, Hruba D, Andel M (2003) Serological markers of *Chlamydia pneumoniae, Cytomegalovirus* and *Helicobacter pylori* infection in diabetic and non-diabetic patients with unstable angina pectoris. Cent Eur J Public Health 11: 102-106.
2. Berger M, Schroder B, Daeschlein G (2000) *Chlamydia pneumoniae* DNA in non-coronary atherosclerotic plaques and circulating leukocytes. J Lab Clin Med 136: 194-200.
3. Blasi F, Boman J, Esposito G (1999) *Chlamydia pneumoniae* DNA detection in peripheral blood mononuclear cells is predictive of vascular infection. J Infect Dis 180: 2074-2076.
4. Christen U, Benke D, Wolfe T (2004) Cure of prediabetic mice by viral infections involves lymphocyte recruitment along an IP-10 gradient. J Clin Invest 113: 74-84.
5. Fong IW, Chiu B, Viira E, Fong MW, Jang D, et al. (1997) Rabbit model for *Chlamydia pneumoniae* infection. J Clin Microbiol 35: 48-52.
6. Hammerschlag MR, Chirgwin K, Roblin PM (1992) Persistent infection with *Chlamydia pneumoniae* following acute respiratory illness. Clin Infect Dis 14: 178-182.
7. Johnson RA (2000) The immune compromised host in the twenty first century: Management of mucocutaneous infections. Semin Cutan Med Surg 19: 19-61.
8. Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, et al. (1980) Breeding of a non-obese, diabetic strain of mice. Jikken Dobutsu 29: 1-13.
9. Muller J, Moller DS, Kjaer M, Nyvad O, Larsen NA, et al. (2003) *Chlamydia pneumoniae* DNA in peripheral blood mononuclear cells in healthy control subjects and patients with diabetes mellitus, acute coronary syndrome, stroke and arterial hypertension. Scand J Infect Dis 35: 704-712.
10. Ossewaard JM, de Vries A, Bestebroer T, Angulo AF (1996) Application of a *Mycoplasma* group specific PCR for monitoring decontamination of *Mycoplasma* infected *Chlamydia* sp. strains. Appl Environ Microbiol 62: 328-331.