Veterinary Immunology and Immunopathology 158 (2014) 30–36

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

What can we learn from the microbial ecological interactions associated with polymicrobial diseases?

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ARTICLE INFO

Keywords:
Polymicrobial disease
Periodontal disease
Microbial ecology
Molecular tools
Anti-infective
Vaccine

ABSTRACT

Periodontal diseases in humans and animals are model polymicrobial diseases which are associated with a shift in the microbial community structure and function; there is therefore a need to investigate these diseases from a microbial ecological perspective. This review highlights three important areas of microbial ecological investigation of polymicrobial diseases and the lessons that could be learnt: (1) identification of disease-associated microbes and the implications for choice of anti-infective treatment; (2) the implications associated with vaccine design and development and (3) application of the dynamics of microbial interaction in the discovery of novel anti-infective agents. This review emphasises the need to invigorate microbial ecological approaches to the study of periodontal diseases and other polymicrobial diseases for greater understanding of the ecological interactions between and within the biotic and abiotic factors of the environment.

1. Introduction

Polymicrobial diseases are recognized as clinical disorders associated with pathologies which are brought about by multiple aetiological agents in complex ecological interactions between and within the abiotic and biotic factors (including host response to the pathogen assault) in environments such as the oral cavity (Brogden et al., 2002; Peters et al., 2012). Such interactions involving bacterial communities have been shown to involve ecological flux (in the community structure and function) characterized by synergistic activities and/or successive replacement of members of the microbial communities in the pathogenesis of the associated disease(s) (Brogden et al., 2002; Jabra-Rizk, 2011). Consequently, these diseases are often referred to as "mixed infections" by medical and veterinary professionals – a term which conceals the true characteristics and hinders in-depth understanding of the nature of these diseases. There are numerous examples of diseases with polymicrobial aetiology, ranging from footrot in sheep to cystitic fibrosis in humans (Brogden et al., 2002). This review focuses on periodontal disease (in humans and animals) as a model for highlighting the benefit of microbial ecological assessment of polymicrobial disease.

Koch's postulates defines a set of rules for the designation of the aetiological agents of diseases based on the ability to culture, inoculate and reisolate a specific microbe which must be able to re-establish the same disease pathology (Evans, 1976). These postulates are readily adapted to those diseases associated with a single aetiological agent. However, little attention has been given to the adaptation of these rules to a polymicrobial disease situation. With increasing availability of molecular tools such as PCR and

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http://dx.doi.org/10.1016/j.vetimm.2013.03.009

nucleic acid hybridization techniques for the detection of microbes and pathogens associated with diseases, the term "Molecular Koch's postulates" was coined (Fredericks and Relman, 1996; Inglis, 2007). This term redefined Koch's postulates to include those pathogens detected, using these modern culture-independent tools (Fredericks and Relman, 1996; Falkow, 1988). The molecular Kock's postulates may aid in the identification of disease-associated microbes in polymicrobial diseases. However, the application of these postulates in polymicrobial disease situations may not be as simple as it is in diseases of single aetiology. This is due to the complex host–pathogen(s) and microbial–microbial interactions involved in the polymicrobial disease processes.

The use of molecular fingerprinting techniques "amplified ribosomal DNA restriction analysis (ARDRA); terminal-restriction fragment length polymorphism (T-RFLP); ribosomal intergenic spacer analysis (IRSA); random amplified polymorphic DNA (RAPD); temperature gradient gel electrophoresis (TGGE); denaturing gradient gel electrophoresis (DGGE); single strand conformation polymorphism (SSCP)" in microbial ecological studies have made it possible to study the changes in microbial community structures and identify the dominant members of the community (Ranjard et al., 2000; Arias et al., 2005). These profiling techniques have been adapted in the study of periodontal diseases which is a well-documented example of polymicrobial disease (Mayrand and Grenier, 1998; Ledder et al., 2007; Ling et al., 2010). These techniques are based on the differential electrophoretic mobility of individual PCR amplicons of the target gene, in an agarose or polyacrylamide gel. The differentiations are either based on size (ARDRA, T-RFLP, 1RSA and RAPD) or sequences (TGGE, DGGE and SSCP) (Ranjard et al., 2000; Dubey et al., 2006). The microbial ecological changes are determined by analysis of the generated molecular fingerprints using multivariate statistical methods which have been extensively described in previous reports (Abdo et al., 2006; Ramette, 2007; Marzorati et al., 2008). It is noteworthy that the degree of resolution of the microbial community structure in a sample is dependent on the sample collection and preparation methods; the efficiency of the PCR amplification of the gene-target and the application of appropriate statistical methods for the analysis and interpretation of result (Ranjard et al., 2000; Dubey et al., 2006; Marzorati et al., 2008).

The major advantages of these methods are (1) affordability as compared to high throughput methods such as the 454 and Illumina sequencing methods (Kowalchuk et al., 2007), (2) the ability to bring to the fore dominant microbial species associated with specific conditions (Ledder et al., 2007) and (3) the relative ease of analysing and interpreting the result using simple statistical tools as compared to those needed for the analysis of complex metagenomic data generated by the high throughput sequencing methods (Kowalchuk et al., 2007). The major limitation of these molecular fingerprint methods is their low resolution, whereby only 0.1–10% of the microbial population in a particular environment can be revealed (Marzorati et al., 2008). Other advantages and limitations of the individual electrophoretic method mentioned above have been extensively discussed elsewhere (Ranjard et al., 2000; Dubey et al., 2006).

Studies have shown that oral diseases are typical models of polymicrobial diseases that involve complex interaction among members of the oral microbiota and the host (Socransky et al., 1998; Marsh, 2003). Specific combinations of these disease-associated microbes have been reported to influence the severity of the pathology observed (Sundqvist et al., 1979). It has also been shown that in some cases, a single member of the community may not be able to reproduce the typical/classical disease but could result in mild pathology which may be self-limiting in nature (Mayrand and McBride, 1980). With the well-established fact that periodontal diseases are a result of complex microbial interactions, it is without doubt that therapeutic/prophylactic protocols in the management of these diseases and other polymicrobial diseases should consider the major players in the pathogenesis of the diseases while also addressing the risk factors that predisposes a host to the diseases.

Microbial interactions associated with the pathogenesis of diseases may be positive (aiding one another for survival and virulence) or negative (antagonistic) and each of these interacting activities form the basis for the establishment of the dominant microbial species (the structural organization) which in turn dictates the functional organization of the microbial community in question (Mayrand and Grenier, 1998). The functional organization directs the pathogenicity of the disease (Siqueira and Rôças, 2009). The structural and functional organizations of such communities may involve interactions such as presentations of receptors for adhesion (Peters et al., 2012); breaking down of complex molecules to ensure nutrient availability to others members of the community (Ramsey et al., 2011); protection of other members from the host immune responses (Gemmell et al., 2004) and potentiating the virulence of the pathogenic members of the community (Saito et al., 2008).

Consequently, it is pertinent that therapeutic/prophylactic strategies should not be limited and/or focussed only, on the major pathogenic members that are able to reproduce disease but also, on those members that play other roles in maintaining the "global structure and function" of the community associated with the disease.

In recognition of the involvement of microbial ecological changes in polymicrobial diseases, other studies have focused on the metabolite profiles of the microbial communities in health and disease and have shown that changes in the microbial community structure are associated with changes in the metabolite profiles (Takahashi, 2005; Takahashi et al., 2010). Such studies suggest that the "global metabolic pathway" assumed by the interacting members of a microbial community are dependent on the prevailing specific conditions of the environment.

Recently, new concepts of periodontal diseases (and other polymicrobial diseases) have been proposed (Hajishengallis et al., 2012; Hajishengallis and Lamont, 2012). These concepts hypothesize the presence of "keystone pathogens" which act to destabilize the host immune defence system thereby enhancing the growth of compatible disease-associated microbes and elevating