The importance of temperature for medical science

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ABSTRACT
Diverse effects of temperature are well-known, from global warming to effects on biochemical reactions. Politically global warming has become a major issue. Body temperature is controlled in a narrow range to maintain normal cellular function. A few degrees change of body temperature impacts significantly on physiological response. The article describes the critical and significant impact of the temperature on medical science research within a microenvironment; on physiological effect of a cell, on conformation of a protein, and on possible benefit of a pathogen, thus urging more attention and research to the temperature effects in research not only on the interpretation of biological results cautiously and on drug development, but also on development of diagnostic reagents.

KEYWORDS: fever, temperature, infectious diseases, medical science

INTRODUCTION
The biological importance of temperature has been well documented while its role in medical science, in the form of fever, has not received appropriate attention. Almost all infectious diseases are associated with fever, and in some infections can be prolonged [1]. Although fever could enhance response to pathogens, other studies have shown that duration of high thermal exposure has been shown to suppress antibody response to viral antigen, induce vascular degeneration and affect physiological function and immune system [2]. Evidence indicates that pathogens have a variety of survival strategies for coping with fever. Fever, therefore, may have a beneficial effect for the pathogen. Biochemical processes are highly sensitive to the changes of temperature. Literature review reveals that majority of reported analytic effectors have not incorporated the corresponding body temperature at the time of specimen collection and during the data analysis [3]. The potential role of fever related parameters, especially body temperature, therefore, has not been investigated thoroughly in medical science and consequently its significance in disease pathogenesis is largely unknown.

Objectives
The importance of the fever in infectious disease has been well established, and yet its role in pathogenesis, as well as in clinical data interpretation, has not been publicized correspondingly. This review is urging more attention of the temperature factor to be incorporated into biomedical research and practice with the following objectives.
1. Describing the impact of environmental temperature changes on the epidemiology of infectious diseases.
2. Addressing the effect of fever on body immunity and the infectious pathogens.
3. Discussing the effect of fever on the biomedical research and performance of diagnostic tests.

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Environmental temperature and infections

Dynamic effects derived from global warming have been noticed [4-6]. The most obvious change is the thinning and shrinkage of the glacier layer [7, 8]. Other changes include insects repopulation of territory where these creatures have not been seen for several decades [9]. The consequences associated with it are likely introduced a new and unfamiliar disease to the new environment [10]. This frequently triggers public concerns [11]. One such example is the spread of *Aedes* spp. mosquitoes, the dominant vector for many arbovirus such as dengue [12, 13]. On the other hand, the rise of the environmental temperature may have a negative impact on some of pathogens that are very sensitive to the temperature changes. For example, coronavirus can be inactivated very rapidly with environmental temperature of >20°C [14] and the survival days of avian influenza virus (H5N1) can be shortened significantly at outdoor temperature >30°C [15]. Thus, the change of environmental temperature could change the epidemiology and patterns of human infectious diseases.

Fever and immunity against infections

Body temperature is regulated by hypothalamic area using several mechanisms to generate necessary heat and dissipate excessive heat. Normal cellular function maintains only in narrow range of temperature, approximately within 0.5-1 degree around 37°C. Fever by definition means “an elevation of body temperature above the normal daily variation” [16], usually defines as body temperature ≥38°C. Although fever may reflect a protective response to any infections or injuries, physiological responses to fever are a double-edged sword to the host, beneficial and harmful [17]. Numerous investigations, though mainly in animal models, support the concept that fever could have a beneficial enhancing the resistance of animals to viral and bacterial infections [18-26]. Evidence of clinical benefit of fever was observed in patients with bacterial sepsis that found a correlation between the temperature and survival [27-29]. Treatment of chicken pox with antipyretic drugs can prolong the duration of pox lesions compared to placebo [30]. Taking antipyretic drugs after immunization can reduce the immunogenicity of pneumococcal vaccine [31].

The interaction of body temperature and viral replication could be complicated as the results of combination of multiple parameters. Dengue virus infection, an important vector-borne human disease, is a good example. The viral burden could be observed steadily increasing at moderate to high fever temperature (38°C to approximately 40°C), and a sudden drop of the viral burden was seen when fever temperature reached at 40°C and beyond (Figure 1A). Dynamic clinical symptoms in dengue, ranging from asymptomatic, undifferentiated fever, dengue fever (DF, normally a self-limited illness), to dengue hemorrhagic fever (DHF) and/or dengue shock syndrome (DSS), characterized by plasma leakage and increased vascular permeability, are well-documented [32]. Viral burden in DF seems to be much more sensitive to fever as evidenced by only a moderate increase of viral burden at elevated temperature and a sudden drop of the viral burden at or around 39.5°C (Figure 1B). In contrast, the patterns of viral burden in DHF were dramatically different from DF. Three noticeable stages of DHF could be observed; a sharp increase of viral burden at moderate fever temperature (38°C-39°C), the steady viral burden at high fever temperature (39°C-40°C), and a significant drop at or beyond abnormal fever temperature (>40°C) (Figure 1B). The reasons are unclear. It may be due to the reduced affinity of antibody to dengue virus at high fever temperature in DHF [32]. Alternatively, antibody may not be able to recognize the dengue virus due to conformational changes in viral antigen resulting from high fever temperature. Additionally, it could be the result of a bystander factor released in secondary infection, promoting a higher efficiency of dengue virus replication in permissive cells.

The underlying mechanisms by which fever affects or controls on the pathogens in a host are unclear, but in general, fever-associated cytokines seem to have major role [33-38]. The pyrogenic cytokines such as tumor necrosis factor-alfa (TNF-α), interleukin (IL)-1, IL-6, and interferon gamma (IFN), are induced by several pathogens [33-38]. For an example, IL-1 could prevent mortality in animals with bacterial sepsis [38].
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Homeostasis and diseases

Biochemical processes are highly sensitive to the changes in temperature. An increase of metabolic rate can be seen when body temperature changes from normal 37°C to 40°C [52]. In fact, a 10 percent increase in metabolic rate with a corresponding 1°C increase of body temperature has been documented [53]. Thus, the rate of metabolism in systemic and/or individual compartment of microenvironment, such as capillary, may increase resulting in an imbalance of homeostasis. Since maintenance of homeostasis is a self adjusted event and is highly operational regulated, any imbalance could result in a disease symptom. The augmentation of the imbalance of homeostasis, in particular, in the blood components of cells in circulation and biological effectors (cytokines) would likely be much more noticeable in such disease condition. The phenotypes expression of such clinical alteration associated with fever in affected patients are lethargy, vomiting, malaise, and to some extent if proper intervention to treat the cause of fever was not initiated immediately or urgently, may lead to more severe conditions; hemorrhage, bleeding in certain organs, and even to a dire consequence, death [51]. Furthermore, the effects of febrile temperature on certain cell types have much more impact than others, which to some extent,
may result in favoring pathogens proliferation. For instance, Japanese encephalitis virus yields are increased by 0.2-2.5 log PFU/ml in heat shocked BHK-21 cultures at 41°C compared to control cultures at 37°C [49]. Flaviviruses in Aedes albopictus cell cultures adapted to 34.5°C is replicative to higher viral titers than at 28°C [50]. In addition, oncolytic virus (adenovirus type 5) can augment the tumor cell killing at fever temperature (39.5°C) [54]. Furthermore, the cold adapted influenza vaccine virus was developed to survive and replicate in cooler sites at anterior nares, and not at 37°C, made it unable to cause systemic infection [55].

**Timing of specimen collection**

Specimens collected for diagnosis, in general, are quite late in the course of illness [1]. Collection of specimens appears to be at the peak of fever, usually at 2-10 days after the onset of infection (Figure 2). In contrast, those samples collected after the clinical symptoms have resolved (4-14 days after infections), are likely during or at the peak of immune response to the pathogens (Figure 2). Results from biological assays with these medical specimens, although informative, may not truly reflect the sequence of events. It is especially implicated in an effort to differentiate the parameters of host responses to the effects of the pathogen’s virulence. Therefore, one would expect that parameters accounting for the cause may be difficult to reach consistency. For instance, in spite of numerous reports on profiling of samples collected from dengue patients to look for a bio-signature or pattern that can be useful in dengue diagnostic value, no consensus or reliable outcome has been reached [56-58]. Bio-signature or biomarker is a biological indicator, either a peptide or an immune profile, obtained by assays of specimens (such as a blood sample) which can be a unique and differentiable bar code for a disease. This scenario can be extended to when a bio-signature or biomarker for a defined disease is the objective of the assays, data mining from these specimens may constitute a bias interpretation of the results.

**Figure 2.** Schematic drawn of sequential events in acute infections. Incubation periods are likely to vary depending upon the nature of the pathogens, mostly 1-7 days in common infections [1] prior to onset of clinical symptoms (fever as an example), which followed by individual’s tolerance level to the clinical symptoms, which can be 1-2 days, before seeking help. The time in which affected individuals seeking help can range from 2-10 days and is likely at the peak of pathogen burden. The duration of the acute infection event can vary but normally would resolve within two weeks, though in some cases, it can last longer than expected. In general, rising levels of immunological, hemostatic or pathophysiological parameters right after the resolving of pathogen burden would be observed.
Biological assay \textit{in vitro} vs. \textit{in vivo}

\textit{In vitro}, most of the biological assays are performed at normal physiological body temperature, 37°C. However, specimens often collected at the time of clinical symptoms are associated with high fever. The high fever could induce alteration of a cell or a protein. The biological assays, such as serum neutralization antibody assay to a pathogen, which is normally performed at 37°C or room temperature \textit{in vitro}, may not produce results reflective of the febrile temperature of the patient. Therefore, the results may not predict or correlate with the events or anticipated outcome \textit{in vivo}. For example, high viremia can be observed in dengue patients in spite of the presence of high neutralizing antibody [59-62]. One might question whether the same neutralizing antibody titer be observed if the assays are performed at febrile temperature such as 39°C or 40°C, and what would be the proper control. These require researchers’ attention and careful experimental designs before a conclusive statement could be drawn.

Fever and evolution of pathogens

Numerous antimicrobial agents are developed to cope with the emergence of resistant organism. Nowadays, drugs are mainly developed and designed based upon crystal structure of a protein or molecule in conjunction with computer modeling [71]. A drug with perfect fit to the space of a computer predicted backbone structure based upon a protein crystallized at 37°C. To some extent, these drugs may have a foreseeable effect on some pathogens initially. But over times, resistance developed [19]. The cause may partially be due to the host immune pressure selection resulting in natural mutation of the pathogens. Other possible causes such as drug fitness should be considered as well, in particular, drugs derived from the crystal structure. Higher temperature may induce dynamic space changes even though overall structure seems to be similar. Thus, drug derived from crystal structure generated at 37°C, may fit pretty well at the normal temperature but may not fit perfectly at the febrile temperature. Consequently, the efficacy of drug treatment may not live up to expectation. Suggestively, this may also potentially induce the selection of pathogen, resistant to the drug.

CONCLUSION

The systemic investigation of the role that temperature plays warrant further research. Especially, in search for specific biomarkers, in structured-based drug design, and perhaps in the re-evaluation of the diagnostic reagents with antigens prepared at different temperatures deserve more study.

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