Heat shock proteins and infection: Interactions of pathogen and host

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Abstract. Invasive microorganisms encounter defensive attempts of the host to starve, destroy and eliminate the infection. In experimental model systems aiming to imitate defensive actions of the host, microorganisms respond by the rapid acceleration in the rate of expression of heat shock and other stress proteins. Heat shock proteins (hsp) of most if not all pathogens are major immune targets for both B- and T-cells. Host cells involved in the defensive action cannot avoid exposure to their own reactive compounds, such as oxygen radicals, resulting in premature cell death and tissue damage. Long-term consequences to the host may include cancer. In cells in tissue culture, induction of host-specific hsps occurs upon exposure to oxidants and in viral infections. Drugs that bind to members of the hsp70 family induce peroxisome proliferation and hepatocarcinoma, but may open the way for the development of novel drugs in support of antimetabolite treatment of infections and cancer.
Key words. Nutrient withholding; phagocytes; oxidants; cancer; peroxisome proliferators.

Introduction

Infectious diseases continue to be a major cause of human morbidity and mortality in economically deprived populations of the Third World [56]. Scientific investigation focuses on an understanding of the dynamic interaction between pathogen and host, thus shedding light on inflammatory and degenerative diseases, which have a less identifiable etiology. Very much in contrast to the pathogens, the commensal microorganisms can be tolerated and fed in huge numbers by their host, often in intimate contact with body surfaces [36].

Whether or not a given pathogen causes an illness, depends on the number of infecting organisms and on the immune status of the host. Extremes of age, inadequate clothing, housing and nutrition, antibiotics and other drugs, and the presence of concomitant diseases are well known to contribute to infectious pathogenesis [22]. Also emotional factors seem to play a role, such as bereavement, divorce, loss of self-esteem [43]. Aarstad and co-workers [1] found changed percentages of spleen B- and CD4-, but not CD8 cells in mice stressed by brief immersion in cold water over a two-week period.

In the light of these results the outsider's position taken by Duesberg [15] appears quite reasonable; he argues that the acquired immunodeficiency syndrome (AIDS), defined by a severe depletion of T cells, cannot be caused by the human immunodeficiency virus (HIV), but by matters of lifestyle such as intravenous drug abuse, frequent transfusions, and promiscuous male homosexual activity.

At all stages of infection, there appears to be an involvement of hsps in both the pathogen and the host. It is the aim of this review to point out the stimuli that lead to an enhanced expression of hsps in both the pathogen and the host and to emphasize the importance of this essentially ubiquitous physiological response.

General features of infectious processes

The pathogen's necessities

Virus-infected bacterial hosts provide the most readily accessible system for viral multiplication and strategies for viral perseverance. During the lytic cycle of a bacteriophage, or of a virus, the infected host cell can become a new entity, completely dominated by the genetic program of the virus to multiply virions. Thus, the synthesis of host-specific components may be inhibited, continued or enhanced due to viral modification of the synthetic machinery of the host. Of interest here is that the level of heat shock protein synthesis is enhanced during the lytic cycle of several viruses. Bacteriophage λ-infected *Escherichia coli* bacteria express heat shock protein GroEL, which is essential for λ virion maturation [14, 28]. GroEL has a high degree of amino acid sequence identity with eukaryotic hsp65, whose level of expression is probably also enhanced in virus-infected animal cells [32]. In addition, the rate of protein synthesis of hsp90 [27] and hsp70 (DnaK) [12, 27, 40] has also been found enhanced in virus-infected animal cells.

Among intracellular non-viral pathogens it is *Mycobacterium leprae, Rickettsia mooseri,* and the trypomastigote form of *Trypanosoma cruzi* [36], which come closest to the host cell by becoming part of the host cell's cytoplasm, where they multiply like a cell organelle that has escaped cellular control. Other intracellular pathogens such as *Mycobacterium tuberculosis, Listeria monocytogenes, Brucella* spp., *Salmonella* spp., and *Leishmania donovani,* always live and multiply in the endosomal compartment, the phagosome, which stems from phagocytosis when the pathogen entered the cell [36]. Further separation from the host's cytoplasm is given by the pathogen's outer cell membrane and cell wall. Thus, the host finds an extra lever, as compared with viruses, to starve that pathogen by limiting supply of nutrients.

Indirect evidence for in vivo starvation comes from our knowledge of the mode of action of sulfonamide drugs.

Sulfonamides inhibit bacteria in vitro only when growing in minimal medium. Supplementation with serine, methionine, thymine and a purine (adenine) allows the bacteria to resume growth[63]. From this, one can conclude that at least one of those four compounds is absent in vivo. Thymine holds a special position in so far as thymine-requiring cell cultures, if deprived of thymine, die the so-called thymineless death. Thymineless death can be induced in bacteria in experimental systems in which thymine-dependent mutants are used and the thymine present in the medium is simply washed away[11,50]. With mammalian cells, thymine deprivation can be induced by the addition of metabolic inhibitors such as 5-fluorodeoxyuridine and trifluorothymidine, drugs that are widely used in cancer chemotherapy[30].

Not all nutrients are in short supply in vivo: *aro* mutants from virulent *Salmonella typhimurium*, and *Bacillus anthracis*, which are growth-dependent on five aromatic compounds in vitro, are attenuated in mouse animal models[21,23,41]. They become virulent again when the diet of the infected mouse is supplemented with just two aromatic compounds, namely *p*-aminobenzoate and 2,3-dihydroxybenzoate, which do not normally occur in the diet of mice. Since growth media for *aro* mutants need the three aromatic amino acids as well, one must conclude that these amino acids are available to the Salmonella *aro* mutants in vivo. *p*-Aminobenzoate is incorporated into dihydropteroic acid, the immediate precursor of folate. This enzymatic reaction is competitively inhibited by sulfonamides, which are structural analogues of *p*-aminobenzoate[63]. 2,3-Dihydroxybenzoate is essential for the synthesis of the siderophore enterochelin, needed to solubilize ferric iron by chelation[21]. Iron, an essential nutrient for all organisms except some lactic acid bacteria[39], is needed in the active centre of enzymes involved in oxygen- and electron transfer. When DNA synthesis depends specifically on a supply of thymine, it also depends on iron, which the ribonucleotide reductases of plants, mammals and a few bacteria need for the synthesis of deoxyribonucleotides, precursors for DNA synthesis. Mammalian cells that have developed resistance against hydroxyurea, an inhibitor that leads to the release of iron from ribonucleotide reductase, showed enhanced levels of ribonucleotide reductase and ferritin, the iron storage protein[35]. It has been claimed by Weinberg[62] that iron-withholding is part of the constitutive defence (natural immunity) in mammals.

Carbon-, nitrogen- or amino acid-deprived *Escherichia coli* bacteria undergo extensive RNA and protein degradation followed by enhanced synthesis of a whole range of proteins[34]. The heat shock proteins DnaK and GroEL are amongst them[34]. Starved *Escherichia coli* bacteria have been shown to be more resistant against heat, ethanol, acid, osmotic and oxidative stress than bacteria adapted to the specific stress stimulus[34]. This resistance of quiescent *Escherichia coli* bacteria is not only reminiscent of some properties of spores that a few bacteria are able to form, but of the general phenomenon that vigorously proliferating cells such as embryonic and tumour cells are more sensitive against metabolic inhibitors and ionising radiation[16]. It is also pertinent to mention here the well-established observation that chemical induction of mutations is much more frequent when cells are in the phase of DNA replication[60].

Phagocytosis, oxygen burst, and microbial response

Inflammation is the constitutive defence to foreign materials. Phagocytes (macrophages and polymorphonuclear leucocytes) take an essential part in the cellular inflammatory response. That includes phagocytosis and killing of the pathogen. Phagocytosis results in the enclosure of the foreign particle in membrane-lined vacuoles[64]. Once this process is complete, degranulation takes place due to fusion of the lysosomal granules with the phagosome, which goes together with the discharge of the biocidal load of the granules into the phagosomes. The granules of polymorphonuclear leucocytes contain peroxidase, lactoferrin, a vitamin B_{12}-binding protein, a variety of cationic proteins, alkaline phosphatase, acid phosphatase, ribonuclease, deoxyribonuclease, nucleotidases, glucuronidase, lysozyme and cathepsins[36]. The first measurable event inside the phagosome is a rapid increase of H^+ ions, as indicated by a fall of pH to 3.5–4.0 within a matter of minutes[36]. This low pH is already sufficient to kill some bacteria; however, a complex antimicrobial program follows this initial acidification, as is indicated by the contents of the lysosomal granules[36]. Starvation of the pathogen for iron is brought about by lactoferrin, which strongly binds iron. Patients deficient in specific lactoferrin-containing granules are subject to recurrent gram-positive and gram-negative bacterial infections[6]. Cationic proteins appear to be bactericidal, and the hydrolases digest and dissolve the invasive material[36]. The most deadly weapons, however, are oxygen and nitrogen radicals[18,19,36,48]. The generation of oxygen radicals is frequently referred to as oxydative burst, because of a simultaneous dramatic increase of cyanide-insensitive oxygen uptake and an increase in pentose phosphate shunt activity[24].

Failure of the human phagocytes to generate those oxidants gives rise to chronic granulomatous disease, bearing the hallmark of recurrent infections and leading to early death[5,55].

Naturally, pathogens have evolved strategies to avoid phagocytic processing. Those strategies can be avoidance of phagocytosis, inhibition of fusion of lysosome with phagosome, or escape from the phagosome into the cytoplasm. The latter strategy is used by viruses, whose envelopes can fuse with the membrane of the phagosome so that the genetic material of the virus is set free in the cytoplasm[36]. Intracellular pathogens make their home either in the cytoplasm or in the phagosome of any suitable cell including macrophages[36].

In attempts to mimic phagocytic oxidative stress, cultured microorganisms have been exposed to oxidants such as hydrogen peroxide or cumene hydroperoxide[9, 37, 54, 61, 69]. Some respond again by the increased expression of either or both of the two major heat shock proteins hsp70 and hsp65[37, 61, 69]. Other heat shock proteins have also been demonstrated to be induced in addition to a new set of proteins specifically inducible by oxidants[37, 61]. In *Listeria monocytogenes* a heat-inducible virulence factor is also, albeit to a lesser extent, induced by hydrogen peroxide[54]. Compounds like ethanol, cadmium and other metal salts, and a variety of drugs also induce heat shock and other proteins[37, 61, 69]. The significance of those unphysiological compounds for probing intracellular in vivo responses is, of course, limited but they help to get some information of the pathogen's responsiveness towards unfavourable conditions and to identify stress proteins that are not heat inducible. *Mycobacterium tuberculosis* enhanced expression of a 69-kDa protein upon exposure to hydrogen peroxide, but neither hsp70 or hsp65 (unpublished results). Furthermore, Flesch and Kaufmann[17] concluded from experimental data that growth inhibition of the close but attenuated relative, *Mycobacterium bovis*, by murine – bone marrow derived – gamma interferon activated macrophages was oxygen independent, but, due to most recent results, dependent on nitrogen radicals[18].

Upon infection of cultured macrophages with *Salmonella typhimurium*, which in vivo reside in macrophages, Buchmeier and Heffron[7] found that the bacteria overexpressed a set of proteins of which they managed to characterize two prominent members. These were again the heat shock proteins DnaK and GroEL. Surprisingly, cultured *Salmonella typhimurium* bacteria overexpress only DnaK (hsp70) on exposure to exogenous hydrogen peroxide[37], endorsing the significance of additional stress factors in intracellular conditions.

In the course of these macrophage infections, some 95% of the intracellular bacteria were killed during the first 5 h following addition of bacteria. Hence, those induced stress proteins which were synthesised according to incorporation of ^{35}S-methionine 1–2 h post-infection were probably from lethally damaged bacteria. It follows that enhanced expression of those proteins does not necessarily save the pathogens from being killed by phagocytes. This experiment, however, directly indicates that hsps are associated with infection.

An indirect but intriguing indication that hsps fulfil prominent functions in infectious processes comes from the observation that hsps from most pathogens are dominant targets to both B- and T-cell immunity[10, 25, 31, 49, 53, 59, 65–68]. This matter is covered in detail by other contributions to this multi-author review.

What defence costs has the host to pay?

Producing oxygen radicals is as dangerous as playing with fire. It is widely accepted that cells are unable to completely prevent those compounds from escaping out of their membranous compartments – such as peroxisomes and phagolysosomes – into the cytoplasm[3, 8, 20]. The spread of endogenous oxidants can get worse with intracellular parasites that live or multiply freely in the cytoplasm, unrestricted by a phagosome membrane. That is the case with viruses and a few other pathogens, e.g. *Mycobacterium leprae* and the others mentioned earlier. Killing of those pathogens needs to sacrifice the whole host cell, either by cytotoxic T-cells or by activated macrophages. Killing by cytotoxic T-cells appears to involve induced osmotic lysis of the infected target cell and perhaps destruction of the viral (and host) DNA by secreted DNase activity[70]. Macrophages, activated by T cell-released lymphokines, secrete bioactive products such as alpha-tumour necrosis factor, proteases, and oxidants on the infected host cell and kill it including the pathogen that it accommodates. The neighbouring cells are likely to be damaged as well[5, 26]. This defence is particularly disadvantageous if the cell cannot be replaced, as is the case in tuberculoid leprosy, where the Schwann cells, that fulfil an essential function in the conductance of the electrical nerve signal in motor neurons, are infected by *Mycobacterium leprae*[26].

The pathogenicity of influenza virus infection in mice has been related to phagocyte-derived oxidants. Oda and co-workers[42] found dramatic protection of mice against influenza virus infection when injected with superoxide dismutase, conjugated to a polymer in order to prolong superoxide dismutase activity in the serum.

Bactericidal action leads the phagocyte to an early death, in spite of the presence of protective enzymes such as superoxide dismutase and catalase in the cytosol of the phagocyte[51, 52].

Like bacteria, mammalian cells respond to exposure to oxidants and agents like ultraviolet radiation, arsenite, cadmium and others by induction of heat shock proteins or by enhancing their level of expression[13, 57]. Polla and co-workers[45] found enhanced expression of hsp70 in human monocytes following hydrogen peroxide exposure. Fever, generally an indicator of infectious diseases, appeared to render polymorphonuclear leukocytes more resistant to heat shock[46], i.e. inhibition of metabolic activity by heat shock was less severe. This is probably due to the well-known phenomenon of thermotolerance[38]. Fever may also provide some cross protection against oxidant exposure, as does a mild heat shock to cultivated monocytes[44]. Hence, fever could be of significance in limiting damage from infection-related endogenous oxidants.

The infection-related damage to the host, either through action of the pathogen directly or by defence-related cell killing and exposure to oxidants, may have long-term consequences. Cell death stimulates proliferation of surviving cells and this appears to be by itself a considerable risk factor for cancer[47]. Ames and Gold[4] and others[33] emphasize that endogenous oxygen radicals massively

damage DNA that can be converted to mutations during cell division [60]. Since mutations arising from DNA damage are multiplied by mitogenesis (induced cell division caused by tissue damage and cell death), it is of no surprise that chronic infections appear to be risk factors for cancer. Accordingly, some infectious diseases are considered established risk factors for cancer, namely those caused by Hepatitis B virus, *Schistosoma haematobium, Schistosoma japonicum, Clonorchis sinensis, Opisthorchis viverrini*, and Epstein Barr virus [47]. *Mycobacterium tuberculosis* is a suggested risk factor for cancer [47]. Lung tumours, suggested to have arisen from old tuberculous lesions, have been described pathologically as 'scar cancers' [58].

On the basis of these results one may speculate that bactericidal action of phagocytes induces heat shock and other proteins within the phagocytes.

Conclusion and outlook

I have gathered data from different aspects of microbial life and host responses that indicate or provide evidence that hsps are involved in infections, both on the part of the pathogen and the host. The conditions that enhance the rate of expression of heat shock proteins are manifold but have in common that they perturb or inhibit cell metabolism.

Selective metabolic inhibition is an aim of therapeutic drug treatment of infectious diseases and cancer [16, 29]. Because of the general involvement of heat shock proteins in the survival and recovery of cells following metabolic perturbance, it is conceivable that heat shock proteins play a crucial role in the treatment with metabolic inhibitors, even though they may not be induced by antimetabolites. Hence they appear to be an additional target for drug treatment, either directly or indirectly by aiming at their genetic regulation. One example for specific binding of a drug to hsp70 is the structurally diverse peroxysome-proliferators, such as clofibrate and nafenopin. They have been recognised to specifically bind to a subset of the eukaryotic hsp70 protein family, near or at the ATP binding site [2]. These compounds exert a predictable response in animals, that is peroxisome proliferation in liver cells with an increase of some peroxisomal enzymes, lowering of serum lipids, induction of ornithine decarboxylase, accumulation of age pigment (lipofuscin), hepatomegaly, and hepatocellular carcinoma [2, 3]. It is unclear whether binding of these drugs renders hsp70 dysfunctional and thus directly triggers peroxisomal proliferation and liver pathophysiology. A drug-binding peroxisome proliferator receptor in responsive tissues has been postulated, to account for the disparity that the drug binding members of hsp70 are abundant in almost all organs of the rat, whereas the response is essentially restricted to the liver [2].

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