Medical Hypotheses 85 (2015) 891–893

Contents lists available at ScienceDirect

Medical Hypotheses

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

Could ehrlichial infection cause some of the changes associated with leukemia, myelodysplastic diseases and autoimmune disorders, and offer antibiotic treatment options?

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ARTICLE INFO

Article history:
Received 2 July 2014
Accepted 12 September 2015

ABSTRACT

We hypothesize that a large group of medical conditions of unknown etiology including leukemia, multiple myeloma, myelodysplastic and autoimmune disorders, may be associated with or caused by an obscure group of intracellular obligate parasitic bacteria named Ehrlichia/Anaplasma (EA). Ensconced in the stem cells of the bone marrow, EA may disrupt the normal development and function of many of the cells of immunity, manifesting itself as different syndromes. Recent studies of the activity of EA suggest direct effects on the immune system consistent with the manifestations of leukemia. We reference here three leukemia patients with direct or indirect evidence of EA infection. Moreover, EA have been shown to be most sensitive to rifamycins. Two moribund leukemia patients with levels of platelets and white cells incompatible with life were treated with therapeutic doses of Rifampin. Though they did not survive, their condition improved dramatically for a time, suggesting Rifampin provided some therapeutic benefit. We assert that these results warrant more extensive study.

Hypothesis: ehrlichial infection and leukemia

Diseases of the immune system broadly described by the term leukemia include acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML). The causes of these leukemic syndromes are unknown though many genetic changes have been associated with some forms.

In leukemia we observe the overproduction of cells needed for immune system function, accompanied by large numbers of immature and dysfunctional cells of immunity (termed blasts) that are inappropriately released into the circulation. Something unknown is causing these blasts to fail to function as expected and accumulate in the system of the patient.

The Ehrlichia/Anaplasma (EA) are a family of obligate intracellular parasitic bacteria that infect leukocytes. They have been recognized as human pathogens for a variety of medical conditions [1,2]. EA can alter the DNA of their host cell during its division, as discussed below. Interference with the normal progression of marrow cell development may facilitate the survival of the bacteria in their host leukocytes, by suppressing apoptosis and could also cause a cascade of subsequent immune system failures.

The EA are a Chlamydia, which have different reproductive methods than many other invasive bacterial pathogens. A study of *Ehrlichia Chaffeensis* infection in a human monocyte cell line demonstrated the ability of EA to alter host genes during transcription (transcriptomic effects) [3]. These effects included suppression of apoptosis, a primary defensive activity of intracellular pathogens regulating cell differentiation, and others essential for survival of the obligatory intracellular parasite. A culture of *Anaplasma phagocytophilum* was induced to grow in human immune system cells and produced most of the changes seen in leukemia [4].

The first reported association of EA with immune system dysfunction dates back to 1973, in a patient with aplastic anemia [5]. Identification of the EA infection required an unusual culture method, as these obligate parasites do not grow in normal microbiologic media [6]. Subsequent reports also link EA with systemic lupus erythematosus (SLE) and myelodysplastic syndromes [7]. Could immune system derangement by parasitic EA link all these syndromes and leukemia as well?

Besides leukemia, the diseases of immunity also include myelodysplastic disease, multiple myeloma, and aplastic anemia. All of these disease states, which cause dysfunction of the immune system, are known to spontaneously metamorphose to the

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http://dx.doi.org/10.1016/j.mehy.2015.09.015
0306-9877/

leukemic state. This indicates that they may be related to a similar or singular infectious agent. Since EA are known to alter the function of immune system cells and have been implicated in many of these diseases, this begs the question of whether EA may be the culprit.

Assuming leukemia is associated with an EA infection of the leukocytes, it would further be expected that treatments impacting the bone marrow would be palliative. This in fact is the case; standard treatment for leukemia includes a large list of substances related to the cells of immunity [8] as well as full bone marrow replacement [9] and often induces a diminution of symptoms for some time, though complete remissions are often rare.

It is further hypothesized, moreover, that treatment of leukemia with antibiotics effective against EA would also result in beneficial impact. This has been tried. The results, cited below [10], hint at proof of EA infection as a cause of leukemia as well as a potentially important course of treatment.

Three illustrative cases

Two of three patients discussed here either presented with leukemia; the other suffered from polycythemia vera, which sometimes progresses to leukemia. Two were treated with Rifampin when all other treatments had been exhausted and the patients were moribund and near death. Though both eventually expired, the dramatic and unexpected improvement in their condition after treatment with Rifampin (an available rifamycin) suggests that EA may have been impacted by the antibiotic. Earlier intervention may have been worthwhile. In the third case, PCR analysis indicated the presence of a previously unknown EA. Taken together these three cases, though not definitive, provide tantalizing evidence of a connection between leukemia and EA [10].

Case number 1

Patient AM was a 64 year old man, who first presented with acute lymphocytic leukemia (ALL). The standard treatment, bone marrow replacement, caused the patient to enter remission for a period of approximately 2 years. Then the leukemia recurred. The patient was sent to home care, being thrombocytopenic and with leukocyte counts below those needed to counter infection. After requesting and receiving treatment with Rifampin, he experienced a rapid increase in platelets and leukocytes, coupled with a dramatic recovery of physical well-being, which lasted for several months. Eventually he developed hearing loss and a rash resembling that seen in cytomegalovirus infection and died suddenly, of unknown causes [10].

Case number 2

MR was a 14 year old male who developed acute myelogenous leukemia (AML). After the early failure of conventional chemotherapy, he developed thrombocytopenia and leukopenia. His white count decreased to less than 200 for 2 months, at which time his mother requested therapeutic doses of Rifampin. Within a short period of treatment, his leukocytes rose from 200 to 24,000, of which 25% were mature. His platelets, however, rose considerably slower. Due to a falling hematocrit, MR was prescribed blood replacement, but he died of blood loss during transfer to a hospital from hospice care [10].

Case number 3

This woman in her 50 s suffered from polycythemia vera, a myelodysplastic disease, which occasionally progresses to leukemia. The patient suddenly developed an acute, rapidly fatal AML. A blood film of her peripheral blood showed unexpected inclusions in her platelets, white cells and red cells. These were morphologically indistinguishable from similar structures found in blood from EA infections. Because she had been splenectomized, her red cells were apparently infected at an observed 2% level. Because of the presumed level of bacteria in her blood, a PCR analysis was done, and showed evidence of a previously unreported Ehrlichia. The details of that PCR are available from the original publication [10].

The rapidly evolving science of study of the EA

The presentation of the factors needed in the study of the relationship of the EA to leukemia includes knowledge of several disciplines, including oncology, infectious disease, immunology and genetics. In Appendix A, we discuss EA-induced changes in the transcriptome and epigenome of human cells, as well as the known molecular similarities between the proliferative states of leukemia and EA infection.

In order for these reports to be understood and become a part of this report, and because these important descriptions of laboratory investigation are unfamiliar to most clinicians engaged in the care of these patients, we included a detailed but carefully presented discussion presented as an Appendix A, Addendum *Vida infra*.

Consequences of the hypothesis

These data do not prove that these apparently unrelated syndromes are caused by the same or similar microorganism, but are so suggestive that much more study of serology and DNA must be done to see if there are enough cases to declare that there is a connection or not. Rifampin is now used for control of Ehrlichia and other pathogens and could easily be the subject of further investigations of its effect on leukemia [11]. The accumulating evidence discussed above gives hope for a significant change in mortality rates from the scourge of leukemia, as well as other autoimmune syndromes. Indeed, application of the antibiotics therapy could be used in a compassionate manner for any leukemia cases that are found to be untreatable with conventional therapy and almost certain to eventually result in death. Any small risk of harm is outweighed by the possibility of help.

Conclusions

Perhaps the mystery of leukemia can be explained as an attack by an unsuspected, hard to detect pathogen, evading destruction by the immune system by infecting, disabling and eliminating the cells and mechanisms of immunity. This is not a new phenomenon, as we know from AIDS and other diseases. The resulting consequence, a flood of dysfunctional immune system cells, presents as the disorder we call leukemia (or in other cases aplastic anemia, polycythemia vera, lupus and similar autoimmune diseases). Leukemia, under this conceptualization, may not be a malignancy at all, but rather an infection that can be treated with antibiotics and other means. Lacking any better explanation for this terrible disease, nor any reliable curative treatments, the possibility that leukemia is caused by or associated with EA or similar infection cries out for more extensive investigation than has taken place to date.

Conflicts of interest

The authors Friedman and Nyindo have no conflict of interest. Author C.A. Kallick has one patent which may have a conflict of interest with some of the information in the paper. Author Kallick has transferred that patent to a partnership composed of his children. Author Kallick has retained only 1% of the possible value of this patent.

Acknowledgements

The authors express gratitude for the following work and assistance; Steven Kallick, Marlyn Twitchell for manuscript and other assistance. To Doctors Mark Lepper, Midrag Ristic, Carl Woese, Stuart Levin and Kathryn Iacono for knowledge, advice, and scientific direction.

Appendix A

Transcriptomic comparison of a human monocyte cell line (THP1) with or without an *E. chaffeensis* (EC) infection elucidated genes that were differentially expressed between the infected and uninfected states. EC infection induces changes in several cellular systems of the monocyte, as evidenced by enrichment of differentially-expressed genes in categories relating to differentiation, apoptosis, and signal transduction. These transcriptomic changes are consistent with a model in which EC inhibits key signaling pathways of human cells, impairing the host cell's internal defensive capabilities and apoptotic potential. This is the predicted high-fitness transcriptomic strategy that a co-evolved intracellular parasite would evolve towards – decrease the ability of the host cell to respond to infection, prevent the host cell from undergoing apoptosis, and encourage the host cell to proliferate at a high rate.

ETV6 is an oncogenic transcription factor involved in hematopoiesis, and mutations in ETV6 are strongly associated with leukemia in children [13]. ETV5 is implicated in malignant cancers [14], and in stem cell proliferation [15]. ETV5 and ETV6 expression levels are altered by EC [12].

BCL proteins and other apoptosis inhibitors are common major driver mutations in acute leukemia at all ages, as gain-of-function mutations in apoptosis inhibitor genes allows a cell to escape internal and/or external regulators of the cell cycle [13]. EC infection in monocytes upregulates the production of several known apoptosis inhibitors, such as BCL2A1, NF-B, BIRC3, IER3, and MCL1 [12].

Akagi et al. [15] use 771 transcriptomic samples of AML to find networks of genes that are differentially utilized in AML. They find that CDK1, CDK2, CDK4, and CDK6 are the most dysregulated central agents in the cell division gene regulatory network, and encourage that these CDKs are the most promising drug available for treatment targets. EC infection of monocytes downregulated CDC2, CDK5, CDK8, and cyclin G1.

Taken together, these mechanistic studies suggest that EC may hijack cellular signaling pathways relating to hematopoietic differentiation, oncogenic malignancy, cell cycle, and pluripotency to promote similar proliferative changes in blood cell phenotype as some leukemias.

Immunohistochemical evidence supports the translocation of the *E. chaffeensis* ankyrin repeat-containing protein p200 into the nucleus of human monocytes during infection [16]. A similar finding has been reported for the translocation of the EC protein TRP120 [17] and another ankyrin repeat-containing protein from *A. phagocytophilum* into the nucleus of human granulocytes [18]. EC p200 proteins are the primary immunoreactive factors in infections of mammalian cells [19], and are involved in cell differentiation decisions and host responses to infection. In the nucleus of infected human cells, chromatin immunoprecipitation followed by microarray quantification (ChIP-chip) against EC p200 revealed that p200 directly interacts with the promoter elements of 200 genes in the human genome. Genes that p200 bound to, and may regulate, were enriched for Gene Ontology (GO) categories of transcriptional regulation and apoptosis [19].

Yeast-two-hybrid screens revealed that EC proteins TRP47, TRP120, and TRP32 physically interact with human proteins [20]. EC proteins interacted with several human genes known to play roles in transcriptional regulation, cell differentiation, apoptosis, and immune response to infection [20]. This evidence adds to a body of literature showing that EC is able to use a variety of mechanisms to reprogram host cells towards uncontrolled proliferation [21].

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