THE 14TH C. L. OAKLEY LECTURE

Candida albicans HSP 90: link between protective and auto immunity

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Summary. Heat shock proteins (HSP) are thought to play a role in the aetiology of autoimmune diseases, but are also common targets for the immune response to many infections. Patients recovering from systemic candidosis produce antibodies to *Candida albicans* HSP 90, both to species-specific epitopes and, more commonly, to epitopes shared with human HSP 90. One such autoreactive antibody was protective in a mouse model of systemic candidosis.

Introduction

Prokaryotic and eukaryotic cells respond to the stress of a sudden rise in temperature by increasing the rate of synthesis of so-called “heat shock proteins” (HSPs).¹ Insults other than heat can induce synthesis of these proteins and it is now clear that many of them are present in normal cells, where they serve important physiological functions. The term “molecular chaperone” has been applied, signifying their more general role as cellular house-keeping proteins.² Autoreactive T cells that react with mycobacterial HSP 65 have been linked to autoimmune diseases, and increased antibodies to HSPs have been observed in rheumatoid arthritis (HSP 65), ankylosing spondylitis (HSP 90) and systemic lupus erythematosus (HSP 70, HSP 90 and ubiquitin).^{3,4} Members of the HSP 60, HSP 70 and HSP 90 families are also major targets for the immune system in infections due to bacteria, protozoa and helminths.⁵ HSP 90, together with its breakdown products, is now recognised as an immunodominant antigen in fungal infections and seroconversion to it is associated with recovery from systemic candidosis.^{6–8} As they are highly conserved, cross-reactive immunity against shared epitopes on HSPs could provide immunity against various different micro-organisms.³ Boosting immuno-reactivity by repeated exposure to microbial HSPs may assure constantly high levels of immunity, bridging the gap between innate and specific immunity.

In this review, it will be shown that autoreactive antibodies to shared epitopes on *Candida albicans* and human HSP 90 are commonly produced in patients

recovering from systemic infection. Cross-reactive antibodies occur in some patients with systemic lupus erythematosus (SLE). These antibodies were protective in a mouse model of systemic candidosis.

The spectrum of candidal infections

C. albicans is a commensal of the oral cavity and gastrointestinal tract, but in immunocompromised individuals it behaves as an opportunist pathogen, giving rise to superficial or systemic infections. Oral and oesophageal candidosis are frequent clinical features of the acquired immune deficiency syndrome (AIDS) but systemic spread is remarkably rare, despite the presence of severe, progressive immunodeficiency.^{9,10} Similarly, dissemination is rare in chronic mucocutaneous candidosis (CMC), a heterogeneous condition in which protracted superficial candidosis is associated with non-lethal defects in T lymphocytes or macrophages.¹¹ In contrast, systemic candidosis is an increasingly common cause of death among immunocompromised and debilitated patients who do not have AIDS.¹² The mortality of this condition, in culture-proven cases, is about 70%.^{13,14}

Because of this very high mortality, some special clinical units with high-risk patients give antifungal agents prophylactically. Occasionally, intensive care or renal units experience an exceptionally high incidence of candidal infections, due to cross-infection between patients with a single strain.^{15,16} Such outbreaks can be recognised by immunoblot fingerprinting or, better still, DNA fingerprinting the isolates collected.^{17,18} If confirmed, existing hand-washing reagents should be replaced with fungicidal disinfectants such as Hibisol® or Betadine® and antifungal prophylaxis should be initiated.¹⁹ With this important exception, most cases of systemic

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candidosis probably arise from invasion by the patient's endogenous strain of yeast.

Immunology

Many arms of the immune system are involved in host defence against *C. albicans* and their relative importance depends in part on whether the infection is superficial or systemic. Defects in cell-mediated immunity (AIDS, CMC and primary immunodeficiency states) are associated with superficial but not systemic candidosis.²⁰ In contrast, mouse models of systemic candidosis have shown the importance of humoral immunity. Athymic mice are no more susceptible to intravenous challenge than euthymic mice.²¹⁻²³ Passive transfer of hyperimmune serum to non-immune mice confers protection, whereas transfer of normal serum or immune lymphocytes does not.^{24, 25}

Serial immunoblots of multiple sera from patients with culture-confirmed systemic candidosis showed that, among patients with detectable antibodies against *C. albicans*, 92% had them to the 47-Kda band, 40% to a 60-Kda band, 27% to a 92-Kda band and 20% to a 40-Kda band.¹⁴ All survivors produced a major antibody response to the 47-Kda band, whereas fatal cases had little or no antibody or falling titres.^{6, 14} The 47-Kda antigen is a heat-stable breakdown product of the larger 92-Kda antigen which circulates in the sera of these patients.^{7, 14} It has been reported independently by two further groups,^{26, 27} and appears to be distinct from the 48-52-Kda enolase antigen described by Buckley and co-workers,^{28, 29} which has a different pattern of antibody reactivity.³⁰ Both the 47-Kda and the 40-Kda antigens have now been identified as breakdown products of the 92-Kda antigen.^{31, 32} Cloning and sequencing revealed that this was HSP 90.⁷ There are probably two HSP 90s in *C. albicans*, one produced constitutively, which on immunoblotting gives rise to the 47-Kda band, and the other heat-inducible, appearing as the 92-Kda band.³¹ Two HSP 90s occur in the yeast *Saccharomyces cerevisiae*, mice and man. In the case of *S. cerevisiae*, both genes are very closely related and appear to have equivalent functions; growth at higher temperatures requires higher concentrations of either protein. Mammalian HSP 90, which shares 60% identity with the *S. cerevisiae* protein, effectively complements the yeast double mutant.³³

Antibody to the 47-Kda antigen was present in patients with CMC and AIDS.^{20, 34} If protective, it would help to explain the rarity with which such patients develop systemic candidosis, despite severe superficial infections. Polyclonal B cell activation may be responsible for the frequency with which this antibody is produced in AIDS patients. In a neonate with candidal meningitis, who recovered, antibody to the 47-Kda antigen was the first to appear in the cerebrospinal fluid.³⁵ The mother's serum contained IgG antibodies to other candidal antigens, but not to the 47-Kda antigen; therefore, the neonate would not

have acquired this potentially protective antibody transplacentally.

The 60-Kda band, the second most commonly recognised in systemically infected patients,¹⁴ is also heat-inducible.³⁶ It may correspond to *S. cerevisiae* HSP 60, which shows extensive sequence homology with bacterial HSP 60.³⁷ Members of the HSP 60 family are major targets for antibodies in many infections and elevated antibodies to mycobacterial HSP 65 have recently been demonstrated in patients with superficial candidosis.³⁸ A monoclonal antibody to the 60-Kda antigen of *C. albicans* was not protective in a mouse model of candidal peritonitis.³⁹ Preliminary attempts to induce protective immunity in mice against leprosy and tuberculosis by immunisation with mycobacterial HSP 65 failed (M. Colston, D. Young, cited by Watson).⁴⁰ Such experiments do not exclude the possibility that humoral or cell-mediated immunity against certain epitopes on HSP 65 can be protective, but further work will be required to determine this.

Epitope mapping and antibody therapy

Sera from infected patients, with antibody to the 47-Kda antigen, were used to map epitopes on the major carboxy fragment of *C. albicans* HSP 90.³² This method, developed by Geysen *et al.*,⁴¹ involves the synthesis of a series of short overlapping peptides on polyethylene rods, which are then probed with patients' sera. It detects only continuous epitopes but has the advantage of being independent of predictive algorithms.

C. albicans HSP 90 shows >50% direct homology with human HSP 90 and 83% direct homology with *S. cerevisiae* HSP 90.⁷ Most of the antibodies produced by these infected patients were directed against highly conserved epitopes, shared with human HSP 90.³² Serum from all the patients recognised epitope C (LKVIK), and epitope B (LSERM) was the second most commonly recognised. Epitope H (DEPAGE), the only *C. albicans*-specific, carboxy terminal epitope, was the third commonest epitope, recognised by about 50% of sera from patients. Therefore, autoreactive antibodies are produced commonly in patients recovering from systemic candidosis, without any clinical evidence of autoimmune sequelae. This is surprising since self-tolerance should lead to conserved epitopes being weakly immunogenic, if at all. However, T cells to self-epitopes on HSP 65 have now been shown to exist in normal individuals.^{42, 43} Clearly, immunity to shared epitopes on HSPs does not necessarily cause autoimmune disease. Genetic background also strongly determines susceptibility and other unknown environmental factors may affect the T cell repertoire available.

Sera from patients with SLE, which occasionally cross-react with human HSP 90,⁴⁴ can also recognise *C. albicans* HSP 90 and its breakdown products at 47 and 40 Kda. The paucity of humoral autoreactivity to

stress proteins argues against a direct role in the pathogenesis of such disorders.⁴⁴

Epitopes C and H were both immunogenic when the synthetic peptide epitope was conjugated to keyhole limpet haemocyanin and used to immunise rabbits.³² The resulting rabbit antisera have been used to develop a serological test for systemic candidosis. In dot-immunobinding assays, these epitope-specific probes are superior in both sensitivity and specificity to unfractionated rabbit antiserum to *C. albicans*.³² They are slightly less sensitive than an affinity-purified rabbit antiserum to the 47-Kda antigen, which recognises multiple epitopes but is technically difficult to prepare.⁴⁵ On theoretical grounds, the rabbit antiserum to epitope H is preferable to that raised against epitope C, since the former will not cross-react with other species of HSP 90.

A murine monoclonal antibody was prepared against the conserved, dominant epitope C. This, together with patients' sera containing this antibody, was examined in a mouse model of systemic candidosis.³⁶ In one of the two patients' sera examined, the only detectable antibody was to the 47-Kda and 40-Kda breakdown products of HSP 90, recognising just epitopes C and H. The monoclonal antibody, given prophylactically, reduced mortality at 24 h from 100% to 66% and, with a less lethal dose of *C. albicans*, from 60% to 20%. The patients' sera, both taken before they received antifungal chemotherapy, halved mortality at 24 h. This protective effect was mediated by the immunoglobulin fraction of the serum. No reduction in mortality was observed with normal human serum, the immunoglobulin fraction thereof, or an irrelevant monoclonal antibody.

The role of HSP 90

Further immunodominant antigens, identified by immunoblotting with infected patients' sera, have now been recognised as HSP 90 homologues in other fungi (*C. parapsilosis* and *Aspergillus fumigatus*) and a gram-positive bacterium, *Corynebacterium jeikeium*.^{8,31} Human HSP 90 is essentially intracellular. It is involved in the assembly and disassembly of proteins into "higher-order structures", and binds to various cellular proteins, keeping them inactive until they have reached their proper intracellular location or have received the correct activation signal. For example, it associates with steroid hormone receptors in their non-ligand-bound state.⁴⁶ In systemic candidosis, a similar, multifunctional HSP 90 circulates in the plasma in large amounts, where it could mimic the biological activities of human HSP 90, but acting in an extracellular rather than an intracellular environment. An example of the pathogenic effects resulting from candidal HSP 90 might be the disseminated intravascular coagulation sometimes associated with

systemic candidosis. HSP 90 is immunologically cross-reactive with prothrombin and both carry the tripeptide VIR.⁴⁷ Antibody to HSP 90 would then be protective because it neutralises these activities. The fungal HSP 90 may be released only when the yeast breaks down, due to autolysis or the host's immune system. This would be comparable to the release of endotoxin during gram-negative septicaemia. Recently, clinical benefit has been demonstrated from the use of a monoclonal antibody to endotoxin in conjunction with antibiotic therapy.⁴⁸

The exact role of human⁴⁹ or fungal HSP 90 in the physiology of the cell remains elusive. In the water mould, *Achlya ambisexualis*, HSP 90 is a component of a steroid hormone receptor complex involved in the regulation of sexual development.⁵⁰ The female strain produces a steroid hormone, antheridiol, which induces both development changes and HSP 90, the non-hormone binding component of the steroid receptor, in the male strain. *C. albicans* reproduces asexually. By analogy, candidal HSP 90 could be a component of a hormone receptor involved in cell to cell interaction leading to germination or budding.

Conclusions

Patients recovering from systemic candidosis invariably seroconvert to *C. albicans* HSP 90, producing antibodies to epitopes shared with human HSP 90. A monoclonal antibody to a dominant conserved epitope was protective in a mouse model of the infection. Immunity to shared epitopes does not itself lead to autoimmune sequelae, since healthy individuals have autoreactive T cells to HSP 65 and a significant proportion of B cells spontaneously producing auto-antibodies to a wide range of self antigens. Cohen and Young have suggested that selected self antigens are dominant because the immunological response to them is already anticipated by preformed regulatory lymphocyte networks.⁵ As a result, the regulatory elements channel the autoimmune response to these dominant self epitopes towards controlled pathways that prevent the development of autoimmune disease.

Human HSP 90 is essentially intracellular, whereas candidal HSP 90 circulates in the plasma in large amounts. It is suggested that antibody is protective because it neutralises the extracellular, fungal HSP 90. In the future we hope to develop a monoclonal antibody to HSP 90 which can be given prophylactically or, in combination with existing antifungal agents, therapeutically.

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References

1. Lindquist S, Craig EA. The heat-shock proteins. *Annu Rev Genet* 1988; **22**: 631-677.
2. Ellis J. Proteins as molecular chaperones. *Nature* 1987; **328**: 378-379.
3. Kaufmann SHE. Heat shock proteins and the immune response. *Immunol Today* 1990; **11**: 129-136.
4. Cohen IR. Microbial heat-shock protein and autoimmune diseases. In: Neu HC (ed) *New antibacterial strategies*. Edinburgh, Churchill Livingstone. 1990: 263-268.
5. Cohen IR, Young DB. Autoimmunity, microbial immunity and the immunological homunculus. *Immunol Today* 1991; **12**: 105-110.
6. Matthews RC, Burnie JP, Tabaqchali S. Immunoblot analysis of the serological response in systemic candidiasis. *Lancet* 1984; **2**: 1415-1418.
7. Matthews RC, Burnie JP. Cloning of a DNA sequence encoding a major fragment of the 47 kilodalton stress protein homologue of *Candida albicans*. *FEMS Microbiol Lett* 1989; **60**: 25-30.
8. Burnie JP, Matthews RC. Heat shock protein 88 and *Aspergillus* infection. *J Clin Microbiol* 1991; **29**: 2099-2106.
9. Reichart CM, Kelly VL, Machert AM. Pathogenic features of AIDS. In: DeVita VT, Hellman S, Rosenberg SA (eds) *AIDS, etiology, diagnosis, treatment, and prevention*. Philadelphia, Lippincott. 1985: 161-184.
10. Eales L-J, Parkin JM. Current concepts in the immunopathogenesis of AIDS and HIV infection. *Br Med Bull* 1988; **44**: 38-55.
11. Kirkpatrick CH, Rich RR, Bennett JE. Chronic mucocutaneous candidiasis: model-building in cellular immunity. *Ann Intern Med* 1971; **74**: 955-978.
12. Edwards JE. Invasive candida infections. Evolution of a fungal pathogen. *N Engl J Med* 1991; **324**: 1060-1062.
13. Horn R, Wong B, Kiehn TE, Armstrong D. Fungemia in a cancer hospital: changing frequency, earlier onset, and results of therapy. *Rev Infect Dis* 1985; **7**: 646-655.
14. Matthews RC, Burnie JP, Tabaqchali S. Isolation of immunodominant antigens from sera of patients with systemic candidiasis and characterisation of serological response to *Candida albicans*. *J Clin Microbiol* 1987; **25**: 230-237.
15. Burnie JP, Odds FC, Lee W, Webster C, Williams JD. Outbreak of systemic *Candida albicans* in intensive care unit caused by cross infection. *BMJ* 1985; **290**: 746-748.
16. Burnie JP, Matthews RC, Lee W *et al.* Four outbreaks of nosocomial systemic candidosis. *Epidemiol Infect* 1987; **99**: 201-211.
17. Lee W, Burnie J, Matthews R. Fingerprinting *Candida albicans*. *J Immunol Methods* 1986; **93**: 177-182.
18. Matthews R, Burnie J. Assessment of DNA fingerprinting for rapid identification of outbreaks of systemic candidiasis. *BMJ* 1989; **298**: 354-357.
19. Burnie JP, Lee W, Williams JD, Matthews RC, Odds FC. Control of an outbreak of systemic *Candida albicans*. *BMJ* 1985; **291**: 1092-1093.
20. Matthews R, Burnie J, Smith D *et al.* Candida and AIDS: evidence for protective antibody. *Lancet* 1988; **2**: 263-266.
21. Cutler JE. Acute systemic candidiasis in normal and congenitally thymic-deficient (nude) mice. *J Reticuloendothel Soc* 1976; **19**: 121-124.
22. Rogers TJ, Balish E, Manning DD. The role of thymus-dependent cell-mediated immunity in resistance to experimental disseminated candidiasis. *J Reticuloendothel Soc* 1976; **20**: 291-298.
23. Giger DK, Domer JE, Moser SA, McQuitty JT. Experimental murine candidiasis; pathological and immune responses in T-lymphocyte-depleted mice. *Infect Immun* 1978; **21**: 729-737.
24. Mourad S, Friedman L. Passive immunisation of mice against *Candida albicans*. *Sabouraudia* 1968; **6**: 103-105.
25. Pearsall NN, Adams BL, Bunni R. Immunologic responses to *Candida albicans*. III. Effects of passive transfer of lymphoid cells or serum on murine candidiasis. *J Immunol* 1978; **120**: 1176-1180.
26. Neale TJ, Muir JC, Drake B. The immunochemical characterisation of circulating immune complex constituents in *Candida albicans* osteomyelitis by isoelectric focusing, immunoblot, and immunoprint. *Aust NZ J Med* 1987; **17**: 201-209.
27. Ferreira RP, Yu B, Niki Y, Armstrong D. Detection of *Candida* antigenuria in disseminated candidiasis by immunoblotting. *J Clin Microbiol* 1990; **28**: 1075-1078.
28. Strockbine NA, Largen MT, Zweibel SM, Buckley HR. Identification and molecular weight characterisation of antigens from *Candida albicans* that are recognised by human sera. *Infect Immun* 1984; **43**: 715-721.
29. Mason AB, Brandt ME, Buckley HR. Enolase activity associated with a *C. albicans* cytoplasmic antigen. *Yeast* 1989; **5** Special issue: S231-S239.
30. Strockbine NA, Largen MT, Buckley HR. Production and characterisation of three monoclonal antibodies to *Candida albicans* proteins. *Infect Immun* 1984; **43**: 1012-1018.
31. Matthews RC. HSP 90, yeasts and *Corynebacterium jeikeium*. *Epidemiol Infect* 1991; **107**: 273-283.
32. Matthews RC, Burnie JP, Lee W. The application of epitope mapping to the development of a new serological test for systemic candidosis. *J Immunol Methods* 1991; **143**: 73-79.
33. Picard D, Khursheed B, Garabedian MJ, Fortin MG, Lindquist S, Yamamoto KR. Reduced levels of hsp 90 compromise steroid receptor action *in vivo*. *Nature* 1990; **348**: 166-168.
34. Burford-Mason AP, Matthews RC, Williams JRB. Transient abrogation of immunosuppression in a patient with chronic mucocutaneous candidiasis following vaccination with *Candida albicans*. *J Infect* 1987; **14**: 147-157.
35. Burnie JP, Matthews RC, Watson JG, Milligan DW, Tabaqchali S. Analysis of the antibody response developing in an infant with *Candida albicans* meningitis. *J Infect* 1986; **12**: 79-83.
36. Matthews RC, Burnie JP, Howat D, Rowland T, Walton F. Auto-antibody to heat shock protein 90 can mediate protection against systemic candidosis. *Immunology* 1991; **74**: 20-24.
37. Reading DS, Hallberg RL, Myers AM. Characterisation of the yeast HSP60 gene coding for a mitochondrial assembly factor. *Nature* 1989; **337**: 655-659.
38. Ivanyi L, Ivanyi J. Elevated antibody levels to mycobacterial 65-kDa stress protein in patients with superficial candidiasis. *J Infect Dis* 1990; **162**: 519-522.
39. Matthews RC, Burnie JP, Fox A *et al.* The diagnostic and therapeutic potential of a monoclonal antibody to the 60-kilodalton nuclear antigen of *Candida albicans*. *Serodiag Immunother* 1989; **3**: 75-86.
40. Watson JD. Leprosy: understanding protective immunity. *Immunol Today* 1989; **10**: 218-221.
41. Geysen HM, Rodda SJ, Mason TJ, Tribbick G, Schoofs PG. Strategies for epitope analysis using peptide synthesis. *J Immunol Methods* 1987; **102**: 259-274.
42. Lamb JR, Bal V, Mendez-Samperio P *et al.* Stress proteins may provide a link between the immune response to infection and autoimmunity. *Int Immunol* 1989; **1**: 191-196.
43. Munk ME, Schoel B, Modrow S, Karr RW, Young RA, Kaufmann SHE. T lymphocytes from healthy individuals with specificity to self-epitopes shared by the mycobacterial and human 65-kilodalton heat shock protein. *J Immunol* 1989; **143**: 2844-2849.
44. Jarjour WN, Jeffries BD, Davis JS, Welch WJ, Mimura J, Winfield J. Autoantibodies to human stress proteins. *Arthritis Rheum* 1991; **34**: 1133-1138.
45. Matthews RC, Burnie J. Diagnosis of systemic candidiasis by an enzyme-linked dot immunobinding assay for a circulating immunodominant 47-kilodalton antigen. *J Clin Microbiol* 1988; **26**: 459-463.
46. Gustafsson J-A, Wikström A-C, Denis M. The non-activated glucocorticoid receptor: structure and activation. *J Steroid Biochem* 1988; **34**: 53-62.
47. Rebbe NF, Ware J, Bertina RM, Modrich P, Stafford DW. Nucleotide sequence of a cDNA for a member of the human 90-kDa heat-shock protein family. *Gene* 1987; **53**: 235-245.
48. Ziegler EJ, Fisher CJ, Sprung CL *et al.* Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. *N Engl J Med* 1991; **324**: 429-436.
49. Hardesty B, Kramer G. The 90,000 dalton heat shock protein, a lot of smoke but no function as yet. *Biochem Cell Biol* 1989; **67**: 749-750.
50. Brunt SA, Riehl R, Silver JC. Steroid hormone regulation of the *Achyla ambisexualis* 85-kilodalton heat shock protein, a component of the *Achyla* steroid receptor complex. *Mol Cell Biol* 1990; **10**: 273-281.