UNIVERSITI PUTRA MALAYSIA

GENERATION AND VALIDATION OF ASPERGILLUS FUMIGATUS MONOCLONAL ANTIBODIES

MAHDI JAFARLOU

FPSK(M) 2009 23

GENERATION AND VALIDATION OF *ASPERGILLUS FUMIGATUS* MONOCLONAL ANTIBODIES

By

MAHDI JAFARLOU

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2009

Specially dedicated to,

My Family

For their unconditional love and support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

GENERATION AND VALIDATION OF *Aspergillus fumigatus* MONOCLONAL ANTIBODIES

By

MAHDI JAFARLOU

July 2009

Chair: Seow Heng Fong, PhD

Faculty: Medicine and Health Sciences

Aspergillus fumigatus is a ubiquitous thermo tolerant fungus associated with a number of diseases in humans and animals. People at high risk for aspergillosis include patients with advanced AIDS, prolonged neutropenia, allogeneic hematopoietic stem cell transplant recipients, solid organ transplant recipients, and chronic granulomatous disease. The diagnosis of invasive fungal infections is difficult, and most mycology laboratories depend on the blood culture system, and the serology diagnosis of invasive aspergillosis has low sensitivity and specificity. Monoclonal antibodies (mAbs) due to their binding specificity, their homogeneity and great ability to be produced in unlimited quantities are important in diagnosis. The main objective of the present research was to develop specific monoclonal antibodies to help in the early detection of invasive aspergillosis.

The hybridoma cells were obtained by fusing *A. fumigatus*–hyperimmunised Balb/c splenocytes with Sp 2/0-Ag14 (Sp2) myeloma cells using polyethylene glycol with

molecular weight of 1450 (PEG 1450). Positive clones were screened by ELISA. Clones with high titre were selected after 4th limiting dilution, and analyzed with Western blotting. ELISA was carried out to determine the isotypes of antibodies. An ELISA based cross-reactiity test was done to examine the cross-reacts of mAbs with other species of *Aspergillus* and *Candida.* The result of isotype test showed that mAbs 1F8, 3B3, 4C6, 4E9, 5B8, 7D7 and 8G3 were $IgG_{1;}$ mAbs 2G10 was IgG_3 and 3B2, 7B2 and 8F4 were IgM and none of the hybridoma clones produced IgA. Kappa (κ) light chains were found in all of the mAbs. Both of mAb 4C6 (97 kDa) and mAb 7D7 (58 kDa) were chosen for *in vivo* production. Mabs 4C6, 7D7 had a strong reactivity with A. *Fumigatus* crude extract and did not show any cross-reactivity with other pathogenic species of *Aspergillus* and *Candida.* The above antibodies displayed a substantial amount of sensitivity and specificity and both of mAbs were able to stain and clearly identify the hyphae and conidia of A. fumigates by immunofluorescence. The study concluded that mAb 4C6 and mAb 7D7 are able to be used as an immunoprobe for recognition of epitopes responsible for shared antigenicity of fungal glycoproteins, for examining their expression during hyphal and conidial morphogenesis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk ijazah Master Sains

PENCIRIAN DAN PENGHASILAN *Aspergillus fumigatus* ANTIBODI MONOKLONAL

Oleh

MAHDI JAFARLOU

Julai 2009

Pengerusi: Seow Heng Fong, PhD

Fakulti: Perubatan dan Sains Kesihatan

Aspergillus fumigatus adalah sejenis kulat yang boleh dijumpai di merata tempat dan mempunyai toleransi terhadap haba. Ia sering dikaitkan dengan beberapa jenis penyakit di kalangan manusia dan haiwan. Orang ramai yang berisiko menhidapi penyakit aspergilosis termasuklah pesakit yang menghidapi penyakit AIDS peringkat lanjut, neutropenia berpanjangan, penerima pemindahan sel stem hematopoetik allogenik, penerima pemindahan organ, dan penyakit granulomatous yang kronik. Diagnosis untuk infeksi kulat invasif adalah sukar dan kebanyakan makmal bergantung pada sistem kultur darah. Walau bagaimanapun, diagnosis serologi untuk aspergilosis invasif mempunyai sensitiviti dan spesifikasi yang rendah. Antibodi monoklonal didapati mempunyai peranan yang sangat penting dalam identifikasi dan rawatan penyakit aspergilosis disebabkan oleh kemampuannya untuk bertindak secara spesifik, kehomogeneitiannya serta kebolehannya untuk dihasilkan dalam kuantiti yang tidak terhingga. Objektif utama kajian ini adalah untuk penghasilan

antibodi monoklonal yang spesifik bagi membantu pengesanan awal penyakit aspergilosis invasif. Kami memperoleh sel hibridoma dengan menyatukan *A. fumigatus*-sel limpa Balb/c dihiperimmunisasi dengan sel myeloma Sp 2/0-Ag14 (Sp2) dengan penggunaan polietilene glycol dengan berat molekular sebanyak 1450 (PEG 1450). Klon positif dipilih menerusi teknik ELISA. Klon-klon dengan nilai titer yang tinggi dipilih selepas pencairan terhad yang ke-4 dan dianalisis dengan ujian Western pemblotan. Teknik ELISA dijalankan untuk menentukan isotaip antibodi yang diperoleh. Ujian menunjukkan bahawa mAbs 1F8, 3B3, 4C6, 4E9, 5B8, 7D7 dan 8G3 adalah $IgG_{1;}$ mAb 2G10 adalah IgG_3 dan 3B2, 7B2 dan 8F4 adalah IgM and tidak ada sebarang klon hibridoma tersebut yang menghasilkan IgA. Rantai ringan Kappa (κ) dijumpai dalam semua mAbs yang dihasilkan. Kedua-dua mAb 4C6 (97 kDa) dan mAb 7D7 (58 kDa) telah dipilih untuk produksi secara *in vivo* dan purifikasi. Ia tidak menunjukkan sebarang tindak balas dengan sepesis lain di kalangan *Aspergillus* dan *Candida.*

ACKNOWLEDGEMENTS

For multiple reasons, this page of acknowledgment is just as important as whole scientific contents of my thesis. First and foremost I would like to thank the professors who have been instrumental in guiding me to attain an M.Sc at Universiti Putra Malaysia.

I am extremely grateful and appreciate my dear supervisor Professor Dr. Seow Heng Fong for giving me the opportunity to be in part of her laboratory and assigning me to conduct my thesis. She has taught me many invaluable lessons in science, research and life; for that I really feel privileged. I want to thank Dr. Rajesh Ramasamy for being my co-supervisor. His friendly pieces of advice and constructive inputs have shaped my thesis project to meet M.Sc standards.

I want to acknowledge the great cooperation and collaboration from Dr. Maha Abdullah., Dr. Sharmili, Dr. Chong Pei Pei, Dr Abdul Rahman Omar, Dr. Behzad Bardarn, Dr. Seyed Reza Moaddab and Dr. Masoumeh Shams; and also my labmates and friends; Mr. Antonysamy, Siti Aishah, Vahid Sarmadi, Dr. Leslie Than, Dr. Jee Jap Meng, Malini Fonseka, Dr. Seyed Yasin Yazdi, Dr. Mandy, Dr. Peyman, Dr. Homa Davoudi, Yian, Choi Ling, Tong , Suik Chen and Wai Yee.

Finally, I would like to thank every member of my family, specially my parents for their unconditional love and supports. They have sacrificed a lot for me and my brothers Javad, Ahmad and Vahid and it is something that I will never forget for the rest of my life, as I will sacrifice just as much for my future students and children.

I certify that an Examination Committee has met on date of viva voice to conduct the final

Universiti Malaya

BUJANG KIM HUAT, PhD
Professor / Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science.

The members of the Supervisory Committee were as follows:

SEOW HENG FONG, PhD
Professor
Department of Pathology
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

RAJESH A/L RAMASAMY, PhD
Lecturer
Department of Pathology
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

HASANAH MOHD.GHAZALI, PhD
Professor / Dean
Scool of Graduate Studies
Universiti Putra Malaysia

Date: 14 January 2010

DECLARATION

I hereby declare that the thesis is based on my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

MAHDI JAFARLOU

Date: 28 July 2009

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADCC	antibody – dependent cell – mediated cytotoxicity
ABTS	2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt
AP	alkaline phosphatase
APS	ammonium persulfate
BSA	bovine serum albumin
$^{\circ}C$	degree Celsius
Cm	Centimeter
CMV	Cytomegalovirus
CO_2	carbon dioxide
dH_2O	distilled water
DMEM	Dulbecco's Modified Eagles' Medium
DMSO	Dimethylsulfoxide
DNA	deoxyribonucleic acid
EBV	Epstein – Barr virus
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme – linked immunosorbent assay
F	fusion (glycoprotein)
FACS	fluorescence activated cell sorter
Fc	fragment crystallisable (Ig)
FBS	fetal bovine serum
g	force of gravity
g	Gram
h	Hour
H_2O_2	hydrogen peroxide
HAT	hypoxanthine, aminopterin and thymidine
HFCS	hybridoma guanine phosphoribosyl transferase

HPLC	high performance liquid chromatography
Ig	Immunoglobulin
Ip	Intraperitoneal
Iv	Intravenous
Kb	Kilobase
L	large (protein)
µg	Microgram
µL	Microlitre
µm	Micrometer
M	Molar
mAb	monoclonal antibody
mAbs	monoclonal antibodies
mg	Milligram
Min	minute (time)
mL	Milliliter
Mm	Millimeter
mM	Millimolar
MOPC	mineral oil plasmacytoma
NA	Neuraminidase
P	Phosphoprotein
pAbs	polyclonal antibodies
PBS	phosphate buffered saline
PBST	phosphate buffered saline – Tween – 20
PEG	polyethylene gloycol
pg	Pictogram
RBC	red blood cell
RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature

S	second (time)
Sc	Subcutaneous
SDS	sodium dodecyl sulphate
SDS –PAGE	sodium dodecyl sulphate – polyacrylamide gel electrophoresis
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
SPP	Species
TMB	3,3',5,5' - tetramethylbenzidine
Tris	Tris – (hydroxymethyl) – aminomethane
UV	Ultraviolet
v/v	volume for volume
w/v	weight for volume

CHAPTER 1

INTRODUCTION

Aspergillus fumigatus is an opportunistic and one of the most ubiquitous of the airborne saprophytic fungi which causes a number of life threatening diseases in humans and animals.

Invasive aspergillosis which occurs in a wide range of clinical scenarios, including allergic aspergillosis, colonization of cavities with or without the formation of a fungus ball (mainly in the lungs, paranasal sinuses, bronchiectasis), acute to chronic necrotizing invasive forms, ocular infections (keratitis), otomycosis, endocarditis, osteomyelitis and skin infections, is protean in its manifestations, and is still associated with an unacceptably high mortality rate. People most at risk for aspergillosis include patients with advanced AIDS, prolonged neutropenia, allogeneic hematopoietic stem cell transplant recipients, solid organ transplant recipients, and chronic granulomatous disease.

Antibodies are important tools used by many investigators in their research and have led to many medical advances. Monoclonal antibodies have been used as essential research tools for multiplicity of research purposes, including Western blotting, immunohistochemistry, immunocyto chemistry, enzyme-linked immunosorbant assay (ELISA), immuno precipitation and flow cytometric analysis. In addition, antibodies are now being designed for therapeutic applications, including suppression of the immune

system after organ transplantation (Koch et al., 2002; Bumgardner et al., 2001a) treatment of cancers such as leukemia and inhibition of angiogenesis (Stephan et al., 2004).

Aspergillosis accounted for almost half of the cases of fungal infections. The diagnosis of invasive fungal infections is difficult and most mycology laboratories depend on the blood culture system. The positive yield of blood culture unfortunately is less than 15% of patients suspected of having invasive fungal infections. Serology diagnosis of invasive aspergillosis has low sensitivity and specificity. Monoclonal antibodies (mAbs) due to their binding specificity, homogeneity and great ability to be produced in unlimited quantities are important in diagnosis. The main objective of the present research is to develop specific monoclonal antibodies to help in the early detection of invasive aspergillosis.

The difficulties of early diagnosis and treatment of fungal infections have given rise to high mortality rate. Indeed, these infections are all too frequently diagnosed at an advanced stage and prone to rapid progression (Segal and Walsh, 2006).

Monoclonal antibodies are produced by cell lines or clones obtained from animals that have been immunized with the substance that is the subject of study. The cell lines are produced by fusing B cells from the immunized animal with myeloma cells. A major advantage of using mAb rather than polyclonal antiserum is the potential availability of almost infinite quantities of a specific monoclonal antibody directed toward a single epitope (Kohler and Milstein, 1975).

In fact, the importance of early and accurate diagnosis of Aspergillus infections and its substantiated pathogenic impact is the main reason to venture into generating monoclonal antibodies.

Current study conducted on (1) culture of *A. fumigatus* ATCC 36607 and extraction of crude antigen to *A. fumigatus,* (2) generate a panel of murine monoclonal antibodies against *A. fumigates* ATCC 36607 (3) *in-vivo* production of monoclones either *in-vivo* and in-vitro, (4) validate the selected hybridomas clones

Specifically, the main objective of this study was to generation specific monoclonal antibodies against *A. fumigatus*, which can be used to develop an accurate and rapid diagnostic test to *aspergillosis.*

REFERENCES

Abarca, M. L., Bragulat, M. R., Castella, G. and Cabanes, F. J. (1994). Ochratoxin A production by strains of Aspergillus niger var. niger. *Appl Environ Microbiol, 60*(7), 2650-2652.

Akerstrom, B. and Bjorck, L. (1986). A physicochemical study of protein G, a molecule with unique immunoglobulin G-binding properties. *J Biol Chem, 261*(22), 10240-10247.

Akerstrom, B., Brodin, T., Reis, K. and Bjorck, L. (1985). Protein G: a powerful tool for binding and detection of monoclonal and polyclonal antibodies. *J Immunol, 135*(4), 2589-2592.

Al-Kattan, K., Ashour, M., Hajjar, W., Salah El Din, M., Fouda, M. and Al Bakry, A. (2001). Surgery for pulmonary aspergilloma in post-tuberculous vs. immuno-compromised patients. *Eur J Cardiothorac Surg, 20*(4), 728-733.

Allo, M. D., Miller, J., Townsend, T. and Tan, C. (1987). Primary cutaneous aspergillosis associated with Hickman intravenous catheters. *N Engl J Med, 317*(18), 1105-1108.

Andriole, V. T. (1993). Invasive aspergillosis: serologic diagnosis. *Recent Results Cancer Res, 132*, 117-125.

Beck-Sague, C., Banerjee, S. and Jarvis, W. R. (1993). Infectious diseases and mortality among US nursing home residents. *Am J Public Health, 83*(12), 1739-1742.

Bennett, F. C. and Yeoman, L. C. (1983). An improved procedure for the 'dot immunobinding' analysis of hybridoma supernatants. *J Immunol Methods, 61*(2), 201-207.

Bennett, J. E. (1995). *Aspergillus species* (4 ed.). New York: Churchill Livingstone.

Bertout, S., Renaud, F., Barton, R., Symoens, F., Burnod, J. and Piens, M. A. (2001). Genetic polymorphism of Aspergillus fumigatus in clinical samples from patients with invasive aspergillosis: investigation using multiple typing methods. *J Clin Microbiol, 39*(5), 1731-1737.

Bjorck, L. and Kronvall, G. (1984). Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. *J Immunol, 133*(2), 969-974.

Bodey, G., Bueltmann, B., Duguid, W., Gibbs, D., Hanak, H. and Hotchi, M. (1992). Fungal infections in cancer patients: an international autopsy survey. *Eur J Clin Microbiol Infect Dis, 11*(2), 99-109.

Boutboul, F., Alberti, C., Leblanc, T., Sulahian, A., Gluckman, E. and Derouin, F. (2002). Invasive aspergillosis in allogeneic stem cell transplant recipients: increasing antigenemia is associated with progressive disease. *Clin Infect Dis, 34*(7), 939-943.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem, 72*, 248-254.

Breen, D. J., Clifton, A. G., Wilkins, P., Uttley, D. and Westmore, G. (1993). Invasive aspergilloma of the skull base. *Neuroradiology, 35*(3), 216-217.

Brodeur, B. R., Tsang, P. and Larose, Y. (1984). Parameters affecting ascites tumour formation in mice and monoclonal antibody production. *J Immunol Methods, 71*(2), 265-272.

Bruce, M. P., Boyd, V., Duch, C. and White, J. R. (2002). Dialysis-based bioreactor systems for the production of monoclonal antibodies--alternatives to ascites production in mice. *J Immunol Methods, 264*(1-2), 59-68.

Bruck, C., Portetelle, D., Glineur, C. and Bollen, A. (1982). One-step purification of mouse monoclonal antibodies from ascitic fluid by DEAE Affi-Gel blue chromatography. *J Immunol Methods, 53*(3), 313-319.

Bumgardner, G. L., Hardie, I., Johnson, R. W., Lin, A., Nashan, B. and Pescovitz, M. D. (2001). *Results of 3-year phase III clinical trials with daclizumab prophylaxis for prevention of acute rejection after renal transplantation. Transplantation, 72*(5), 839-845.

Burchiel, S. W., Billman, J. R. and Alber, T. R. (1984). Rapid and efficient purification of mouse monoclonal antibodies from ascites fluid using high performance liquid chromatography. *J Immunol Methods, 69*(1), 33-42.

Calera, J. A., Ovejero, M. C., Lopez-Medrano, R., Segurado, M., Puente, P. and Leal., F. (1997). Characterisation of the Aspergillus nidulans aspnd1 gene demonstrates that the ASPND1 antigen, which it encodes, and several Aspergillus fumigatus immunodominant antigens belong to the same family. *Infect Immun, 65*(4), 1335-1344.

Callister, M. E., Griffiths, M. J., Nicholson, A. G., Leonard, A., Johnson, E. M. and Polkey, M. I. (2004). A fatal case of disseminated aspergillosis caused by a non-sporulating strain of Aspergillus fumigatus. *J Clin Pathol, 57*(9), 991-992.

Cariello, N. F. and Skopek, T. R. (1993). In vivo mutation at the human HPRT locus. *Trends Genet, 9*(9), 322-326.

Cenci, E., Perito, S., Enssle, K. H., Mosci, P., Latge, J. P. and Romani, L. (1997). Th1 and Th2 cytokines in mice with invasive aspergillosis. *Infect Immun, 65*(2), 564-570.

Chander, J., Chakrabarti, A., Sharma, A., Saini, J. S. and Panigarhi, D. (1993). Evaluation of Calcofluor staining in the diagnosis of fungal corneal ulcer. *Mycoses, 36*(7-8), 243-245.

Chandler FW., W. Kaplan and L. Ajello. 1980. A colour atlas and textbook of the histopathology of mycotic diseases. Wolfe Medical Publications Ltd. London.

Chandrasekar, P. H., G. Alangaden, and E. Manavathu. (2000). Aspergillosis: an increasing problem in tertiary care hospitals? Clin. Infect. Dis. 30:984-985.

Chazalet, V., Debeaupuis, J. P., Sarfati, J., Lortholary, J., Ribaud, P. and Shah, P. (1998). Molecular typing of environmental and patient isolates of Aspergillus fumigatus from various hospital settings. *J Clin Microbiol, 36*(6), 1494-1500.

Chen, E. H. and Olson, E. N. (2005). Unveiling the mechanisms of cell-cell fusion. *Science, 308*(5720), 369-373.

Choi, J. K., Mauger, J. and McGowan, K. L. (2004). Immunohistochemical detection of Aspergillus species in pediatric tissue samples. *Am J Clin Pathol, 121*(1), 18-25.

Christin, L., Wysong, D. R., Meshulam, T., Hastey, R., Simons, E. R. and Diamond, R. D. (1998). Human platelets damage Aspergillus fumigatus hyphae and may supplement killing by neutrophils. *Infect Immun, 66*(3), 1181-1189.

Coffino, P., Baumal., R., Laskov, R. and Scharff, M. D. (1972). Cloning of mouse myeloma cells and detection of rare variants. *J Cell Physiol, 79*(3), 429-440.

Coleclough, C., Perry, R. P., Karjalainen, K. and Weigert, M. (1981) *Nature* 290: 372-78.

Cordonnier, C., Gilain, L., Ricolfi, F., Deforges, L., Girard-Pipau, F. and Poron, F. (1996). Acquired ciliary abnormalities of nasal mucosa in marrow recipients. *Bone Marrow Transplant, 17*(4), 611-616.

Costa, C., Costa, J. M., Desterke, C., Botterel, F., Cordonnier, C. and Bretagne, S. (2002). Real-time PCR coupled with automated DNA extraction and detection of galactomannan antigen in serum by enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis. *J Clin Microbiol, 40*(6), 2224-2227.

Dangl, J. L. and Herzenberg, L. A. (1982). Selection of hybridomas and hybridoma variants using the fluorescence activated cell sorter. *J Immunol Methods, 52*(1), 1-14.

Davis, J. M., Pennington, J. E., Kubler, A. M. and Conscience, J. F. (1982). A simple, single-step technique for selecting and cloning hybridomas for the production of monoclonal antibodies. *J Immunol Methods, 50*(2), 161-171.

De Repentigny, L., S., Petitbois, M., Boushira, E., Michaliszyn, S., Senechal., N. and Gendron. (1993). Acquired immunity in experimental murine aspergillosis is mediated by macrophages. *Infect Immun, 61*, 3791– 3802.

De Vos, M. M. and Nelis, H. J. (2003). Detection of Aspergillus fumigatus hyphae by solid phase cytometry. *J Microbiol Methods, 55*(3), 557-564.

DeHart, D. J., Agwu, D. E., Julian, N. C. and Washburn, R. G. (1997). Binding and germination of Aspergillus fumigatus conidia on cultured A549 pneumocytes. *J Infect Dis, 175*(1), 146-150.

Denning, D. W., Kibbler, C. C. and Barnes, R. A. (2003). British Society for Medical Mycology proposed standards of care for patients with invasive fungal infections. *Lancet Infect Dis, 3*(4), 230-240.

Denning, D. W. (1996a). Aspergillosis: diagnosis and treatment. *Int J Antimicrob Agents, 6*(3), 161-168.

Denning, D. W. (1996b). Therapeutic outcome in invasive aspergillosis. *Clin Infect Dis, 23*(3), 608-615.

Dixon, D. M. and Walsh, T. J. (1992). Human pathogenesis. *Biotechnology, 23*, 249-267.

Duarte IG, Gal AA. and Mansour KA. (1998). Pathologic findings in lung volume reduction surgery. *Chest*;113:660-664.

Falkenberg, F. W., Weichert, H., Krane, M., Bartels, I., Palme, M. and Nagels, H. O. (1995). *In vitro* production of monoclonal antibodies in high concentration in a new and easy to handle modular minifermenter. *J Immunol Methods, 179*(1), 13-29.

Fink, J. N. and Zacharisen, M. C. (2003). *Hypersensitivity pneumonitis* (Principles and Practice, Trans. 2nd edn ed.). Philadelphia: Mosby.

Fontes, L. V., Campos, G. S., Beck, P. A., Brandao, C. F. and Sardi, S. I. (2005). Precipitation of bovine rotavirus by polyethylene [corrected] glycol (PEG) and its application to produce polyclonal and monoclonal antibodies. *J Virol Methods, 123*(2), 147-153.

Fujimura, M., Ishiura, Y., Kasahara, K., Amemiya, T., Myou, S. and Hayashi, Y. (1998). Necrotizing bronchial aspergillosis as a cause of hemoptysis in sarcoidosis. *Am J Med Sci, 315*(1), 56-58.

Gagnon, G. and Raymond, Y. (1985). Cloning of hybridomas by a single-cell transfer technique. *J Immunol Methods, 78*(2), 267-269.

Galfre, G., Howe, S. C., Milstein, C., Butcher, G. W. and Howard, J. C. (1977). Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature, 266*(5602), 550-552.

Galfre, G. and Milstein, C. (1981). Preparation of monoclonal antibodies: strategies and procedures. *Methods Enzymol, 73*(Pt B), 3-46.

Gangneux, J. P., Bretagne, S., Cordonnier, C., Datry, A., Derouin, F. and Grillot, R. (2002). Prevention of nosocomial fungal infection: the French approach. *Clin Infect Dis, 35*(3), 343-346.

Garcia-Gonzalez, M., Bettinger, S., Ott, S., Olivier, P., Kadouche, J. and Pouletty, P. (1988). Purification of murine IgG3 and IgM monoclonal antibodies by euglobulin precipitation. *J Immunol Methods, 111*(1), 17-23.

Garcia, M. E., Blanco, J. L., Caballero, J. and Gargallo-Viola, D. (2002). Anticoagulants interfere with PCR used to diagnose invasive aspergillosis. *J Clin Microbiol, 40*(4), 1567-1568.

Gefter, M. L., Margulies, D. H. and Scharff, M. D. (1977). A simple method for polyethylene glycol-promoted hybridization of mouse myeloma cells. *Somatic Cell Genet, 3*(2), 231-236.

Gefter, W. B., Albelda, S. M., Talbot, G. H., Gerson, S. L., Cassileth, P. A. and Miller, W. T. (1985). Invasive pulmonary aspergillosis and acute leukemia. Limitations in the diagnostic utility of the air crescent sign. *Radiology, 157*(3), 605-610.

Gerson, S. L., Talbot, G. H., Lusk, E., Hurwitz, S., Strom, B. L. and Cassileth, P. A. (1985). Invasive pulmonary aspergillosis in adult acute leukemia: clinical clues to its diagnosis. *J Clin Oncol, 3*(8), 1109-1116.

Goding, J. W. (1980). Antibody production by hybridomas. *J Immunol Methods, 39*(4), 285-308.

Goding, J. w. (1996). *Introduction to Monoclonal Antibodies.* . london: Academic Press.

Gordon, S. (1975). Cell fusion and some subcellular properties of heterokaryons and hybrids. *J Cell Biol, 67*(2PT.1), 257-280.

Grocott, R. G. (1955). A stain for fungi in tissue sections and smears using Gomori's methenamine-silver nitrate technic. *Am J Clin Pathol, 25*(8), 975-979.

Hales, A. (1977). A procedure for the fusion of cells in suspension by means of polyethylene glycol. *Somatic Cell Genet, 3*(2), 227-230.

Harlow, J. R. and Lane, D. (1988). *Antibodies: A Laboratory Manual*. New York: Cold Spring Harbour Laboratory.

Harkins, K. R. (2001). Antibody purification methods. In Basic Methods in antibody Production and Characterisation, ed. Howard, G. C. and Bethell, D.R.,pp 141-168. Boca Raton: CRC Press.

Harrach, B. and Robenek, H. (1991). Immunocytochemical localization of apolipoprotein A-I using polyclonal antibodies raised against the formaldehyde-modified antigen. *J Microsc, 161*(Pt 1), 97-108.

Hayden, R. T., Isotalo, P. A., Parrett, T., Wolk, D. M., Qian, X. and Roberts, G. D. (2003). In situ hybridization for the differentiation of Aspergillus, Fusarium, and Pseudallescheria species in tissue section. *Diagn Mol Pathol, 12*(1), 21-26.

Hendolin, P. H., Paulin, L., Koukila-Kahkola, P., Anttila, V. J., Malmberg, H. and Richardson, M. (2000). Panfungal PCR and multiplex liquid hybridization for detection of fungi in tissue specimens. *J Clin Microbiol, 38*(11), 4186-4192.

Hetherington, S. V., Henwick, S., Parham, D. M. and Patrick, C. C. (1994). Monoclonal antibodies against a 97-kilodalton antigen from Aspergillus flavus. *Clin Diagn Lab Immunol, 1*(1), 63-67.

Hieter, P. A., Korsmeyer, S. J., Waldmann, T. A and Leder, P. (1981) *Nature* 290: 368-72

Hoogenraad, N., Helman, T. and Hoogenraad, J. (1983). The effect of pre-injection of mice with pristane on ascites tumour formation and monoclonal antibody production. *J Immunol Methods, 61*(3), 317-320.

Hopwood, V., Johnson, E. M., Cornish, J. M., Foot, A. B., Evans, E. G. and Warnock, D. W. (1995). Use of the Pastorex aspergillus antigen latex agglutination test for the diagnosis of invasive aspergillosis. *J Clin Pathol, 48*(3), 210-213.

Horvath, J. A. and Dummer, S. (1996). The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. *Am J Med, 100*(2), 171-178.

Ishihara, T. and Yamamoto, S. (2005). Optimisation of monoclonal antibody purification by ion-exchange chromatography. Application of simple methods with liear gradient elution experimental data. *Journal of Chromatography A, 1069*(1), 77-87.

Iwen, P. C., Rupp, M. E., Langnas, A. N., Reed, E. C. and Hinrichs, S. H. (1998). Invasive pulmonary aspergillosis due to Aspergillus terreus: 12-year experience and review of the literature. *Clin Infect Dis, 26*(5), 1092-1097.

Kaufman, L., Standard, P. G., Jalbert, M. and Kraft, D. E. (1997). Immunohistologic identification of Aspergillus spp. and other hyaline fungi by using polyclonal fluorescent antibodies. *J Clin Microbiol, 35*(9), 2206-2209.

Kearney, J. F., Radbruch, A., Liesegang, B. and Rajewsky, K. (1979). A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. *J Immunol, 123*(4), 1548-1550.

Kincade, P. W., Lee, G., Sun, L. and Watanabe, T. (1981). Monoclonal rat antibodies to murine IgM determinants. *J Immunol Methods, 42*(1), 17-26.

Klebe, R. J. and Mancuso, M. G. (1982). Enhancement of polyethylene glycol-mediated cell hybridization by inducers of erythroleukemia cell differentiation. *Somatic Cell Genet, 8*(6), 723-730.

Klich, M. A. (2002). *Identification of common Aspergillus species.* Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures.

Koch, M., Niemeyer, G., Patel, I., Light, S. and Nashan, B. (2002). Pharmacokinetics, pharmacodynamics, and immunodynamics of daclizumab in a two-dose regimen in liver transplantation. *Transplantation, 73*(10), 1640-1646.

Kohler, G., Pearson, T. and Milstein, C. (1977). Fusion of T and B cells. *Somatic Cell Genet, 3*(3), 303-312.

Kohler, G. and Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature, 256*(5517), 495-497.

Kontoyiannis, D. P., Lewis, R. E., May, G. S., Osherov, N. and Rinaldi, M. G. (2002). Aspergillus nidulans is frequently resistant to amphotericin B. *Mycoses, 45*(9-10), 406-407.

Kozel, T. R. (1996). Activation of the complement system by pathogenic fungi. *Clin Microbiol Rev, 9*(1), 34-46.

Kreutz, F. T., Wishart, D. S. and Suresh, M. R. (1998). Efficient bispecific monoclonal antibody purification using gradient thiophilic affinity chromatography. *J Chromatogr B Biomed Sci Appl, 714*(2), 161-170.

Kwon-Chung, K. J., Bennett, J. E. and Philadelphia. (1992). *Medical mycology.*: Lea and Febiger.

Lane, R. D. (1985). A short-duration polyethylene glycol fusion technique for increasing production of monoclonal antibody-secreting hybridomas. *J Immunol Methods, 81*(2), 223-228.

Latge, J. P. (1999). Aspergillus fumigatus and aspergillosis. *Clin Microbiol Rev, 12*(2), 310-350.

Levitz, S. M. and Farrell, T. P. (1990). Human neutrophil degranulation stimulated by Aspergillus fumigatus. *J Leukoc Biol, 47*(2), 170-175.

Liddel, J. E. and Cryser, A. (1991). Myeloma cells, lymphocytes, fusion. In *A Practical Guide to Monoclonal Antibodies,* pp 67- 88. Chichester: John Wiley and Sons.

Lietzke, R. and Unsicker, K. (1985). A statistical approach to determine monoclonality after limiting cell plating of a hybridoma clone. *J Immunol Methods, 76*(2), 223-228.

Littlefield, J. W. (1964). Selection of Hybrids from Matings of Fibroblasts in Vitro and Their Presumed Recombinants. *Science, 145*, 709-710.

Loeffler, J., Hebart, H., Brauchle, U., Schumacher, U. and Einsele, H. (2000). Comparison between plasma and whole blood specimens for detection of Aspergillus DNA by PCR. *J Clin Microbiol, 38*(10), 3830-3833.

Lyon, M. F. (1961). Gene action in the X-chromosome of the mouse (Mus musculus L.). *Nature, 190*, 372-373.

Madan, T., Eggleton, P., Kishore, U., Strong, P., Aggrawal., S. S. and Sarma, P. U. (1997). Binding of pulmonary surfactant proteins A and D to Aspergillus fumigatus conidia enhances phagocytosis and killing by human neutrophils and alveolar macrophages. *Infect Immun, 65*(8), 3171-3179.

Maizels, R. M., Burke., J. and Sutanto., I. (1987). Phosphorycholine- bearing antigens in filarial nematode parasites: analysis of somatic extracts, *in vivo* secretions and infection sera from Brugia malayi and B. pahangi. *Parasite Immunol, 9*, 49-66.

Martinelli, S. D. (1994). Aspergillus: 50 years on. *Prog Ind Microbiol, 29*, 1-851.

Marusich, M. F. (1988). Efficient hybridoma production using previously frozen splenocytes. *J Immunol Methods, 114*(1-2), 155-159.

Marx, U., Embleton, M.J., Fischer, R., Gruber, F.P., Hansson, H., Heuer, J., de Leeuw, T., Logtenberg, W., Merz, D. Portetelle, J.L,. Romette, D. and Straughan, W. (1997). *Monoclonal antibody production: The report and recommendations of ECVAM Workshop* ATLA: ECVAM.

McCarty, J. M., Flam, M. S., Pullen, G., Jones, R. and Kassel, S. H. (1986). Outbreak of primary cutaneous aspergillosis related to intravenous arm boards. *J Pediatr, 108*(5 Pt 1), 721-724.

McKearn, T. J., Fitch, F. W., Smilek, D. E., Sarmiento, M. and Stuart, F. P. (1979). Properties of rat anti-MHC antibodies produced by cloned rat-mouse hybridomas. *Immunol Rev, 47*, 91-115.

McKinney, M. M. and Parkinson, A. (1987). A simple, non-chromatographic procedure to purify immunoglobulins from serum and ascites fluid. *J Immunol Methods, 96*(2), 271-278.

Melo, JL, Hawkins, R. and Pepys, J. (1977). Studies in chronic bronchopulmonary aspergillosis: 1. Clinical and physiological findings. *Thorax,* 3,257-261.

Mercer, W. E. and Schlegel, R. A. (1979). Phytohemagglutinin enhancement of cell fusion reduces polyethylene glycol cytotoxicity. *Exp Cell Res, 120*(2), 417-421.

Merz, W. G. and Roberts, G. D. (1999). *Algorithims for detection and identification of fungi.* Paper presented at the Manual of clinical microbiology, 7th edn, Washington DC.

Meshulam, T., Levitz, S. M., Christin, L. and Diamond, R. D. (1995). A simplified new assay for assessment of fungal cell damage with the tetrazolium dye, (2,3)-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanil ide (XTT). *J Infect Dis, 172*(4), 1153-1156.

Miyahara, M., Nakamura, H. and Hamaguchi, Y. (1984). Colcemid treatment of myeloma prior to cell fusion increases the yield of hybridomas between myeloma and splenocyte. *Biochem Biophys Res Commun, 124*(3), 903-908.

Moro, A. M., Rodrigues, M. T., Gouvea, M. N., Silvestri, M. L., Kalil, J. E. and Raw, I. (1994). Multiparametric analyses of hybridoma growth on glass cylinders in a packed-bed bioreactor system with internal aeration. Serum-supplemented and serum-free media comparison for MAb production. *J Immunol Methods, 176*(1), 67-77.

Morrison, V. A., Haake, R. J. and Weisdorf, D. J. (1993). The spectrum of non-Candida fungal infections following bone marrow transplantation. *Medicine (Baltimore), 72*(2), 78-89.

Mueller, U. W., Hawes, C. S. and Jones, W. R. (1986). Monoclonal antibody production by hybridoma growth in Freund's adjuvant primed mice. *J Immunol Methods, 87*(2), 193-196.

Mullbacher, A. and Eichner, R. D. (1984). Immunosuppression *in vitro* by a metabolite of a human pathogenic fungus. *Proc Natl Acad Sci U S A, 81*(12), 3835-3837.

National Committee for Clinical Laboratory Standards. (2002) *Reference Methods for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi.*

Approved guideline M38-A. National Committee for Clinical Laboratory Standards. Wayne, Pa.

Neoh, S. H., Gordon, C., Potter, A. and Zola, H. (1986). The purification of mouse monoclonal antibodies from ascitic fluid. *J Immunol Methods, 91*(2), 231-235.

Netea, M. G., Warris, A., Van der Meer, J. W., Fenton, M. J., Verver-Janssen, T. J. and Jacobs, L. E. (2003). Aspergillus fumigatus evades immune recognition during germination through loss of toll-like receptor-4-mediated signal transduction. *J Infect Dis, 188*(2), 320-326.

Nierman, W. C., May, G., Kim, H. S. anderson, M. J., Chen, D. and Denning, D. W. (2005). What the Aspergillus genomes have told us. *Med Mycol, 43 Suppl 1*, S3-5.

Nolard, N. (1994). [Links between risks of aspergillosis and environmental contamination. Review of the literature]. *Pathol Biol (Paris), 42*(7), 706-710.

Norris, B. J., Gramer, M. J. and Hirschel, M. D. (2001). *Growth of cell line in bioreactors.*: Boca Raton:CRC Press.

Norwood, T. H., Zeigler, C. J. and Martin, G. M. (1976). Dimethyl sulfoxide enhances polyethylene glycol-mediated somatic cell fusion. *Somatic Cell Genet, 2*(3), 263-270.

Osmani, S. A. and Mirabito, P. M. (2004). The early impact of genetics on our understanding of cell cycle regulation in Aspergillus nidulans. *Fungal Genet Biol, 41*(4), 401-410.

Patel, R. and Brown, J. C. (1984). Hybridoma preservation at -70 degrees C: a simple and economical procedure for the short-term storage and individual recovery of hybridomas. *J Immunol Methods, 71*(2), 211-215.

Patel, R. and Paya, C. V. (1997). Infections in solid-organ transplant recipients. *Clin Microbiol Rev, 10*(1), 86-124.

Phillips, P. and Weiner, M. H. (1987). Invasive aspergillosis diagnosed by immunohistochemistry with monoclonal and polyclonal reagents. *Hum Pathol, 18*(10), 1015-1024.

Pitt, J. I. (1994). The current role of Aspergillus and Penicillium in human and animal health. *J Med Vet Mycol, 32 Suppl 1*, 17-32.

Potter, M. (1972). Immunoglobulin-producing tumors and myeloma proteins of mice. *Physiol Rev, 52*(3), 631-719.

Prior, J. L. and Titball, R. W. (2002). Monoclonal antibodies against Yersinia pestis lipopolysaccharide detect bacteria cultured at 28 degrees C or 37 degrees C. *Mol Cell Probes, 16*(4), 251-256.

Rantakokko-Jalava, K., Laaksonen, S., Issakainen, J., Vauras, J., Nikoskelainen, J. and Viljanen, M. K. (2003). Semiquantitative detection by real-time PCR of Aspergillus fumigatus in bronchoalveolar lavage fluids and tissue biopsy specimens from patients with invasive aspergillosis. *J Clin Microbiol, 41*(9), 4304-4311.

Reverberi, M., Zjalic, S., Ricelli, A., Punelli, F., Camera, E. and Fabbri, C. (2008). Modulation of antioxidant defense in Aspergillus parasiticus is involved in aflatoxin biosynthesis: a role for the ApyapA gene. *Eukaryot Cell, 7*(6), 988-1000.

Ribaud, P., Chastang, C., Latge, J. P., Baffroy-Lafitte, L., Parquet, N.and Devergie, A. (1999). Survival and prognostic factors of invasive aspergillosis after allogeneic bone marrow transplantation. *Clin Infect Dis, 28*(2), 322-330.

Richardson, M. D. and Patel, M. (1995). Stimulation of neutrophil phagocytosis of Aspergillus fumigatus conidia by interleukin-8 and N-formylmethionyl-leucylphenylalanine. *J Med Vet Mycol, 33*(2), 99-104.

Richardson, M. D., Shankland, G. S. and Gray, C. A. (1991). Opsonizing activity of C-reactive protein in phagocytosis of Aspergillus fumigatus conidia by human neutrophils. *Mycoses, 34*(3-4), 141-143.

Roder, J. C., Cole, S. P. and Kozbor, D. (1986). The EBV-hybridoma technique. *Methods Enzymol, 121*, 140-167.

Roilides, E., Uhlig, K., Venzon, D., Pizzo, P. A. and Walsh, T. J. (1993). Enhancement of oxidative response and damage caused by human neutrophils to Aspergillus fumigatus hyphae by granulocyte colony-stimulating factor and gamma interferon. *Infect Immun, 61*(4), 1185-1193.

Romani, L. (2004). Immunity to fungal infections. *Nat Rev Immunol, 4*(1), 1-23.

Ruchel, R. and Schaffrinski, M. (1999). Versatile fluorescent staining of fungi in clinical specimens by using the optical brightener Blankophor. *J Clin Microbiol, 37*(8), 2694-2696.

Russell, P. H. and Alexander, D. J. (1983). Antigenic variation of Newcastle disease virus strains detected by monoclonal antibodies. *Arch Virol, 75*(4), 243-253.

Samson, R. A., Houbraken J., Summerbell RC., Flannigan B. and Miller JD (2001). *Common and important species of fungi and actinomycetes in indoor environments*. New York: Taylor and Francis.

Samson, R. A., Jos A.M.P. Houbraken1, Angelina F.A. Kuijpers, J. Mick Frank and Jens C. Frisva (2004). New ochratoxin A or sclerotium producing species in Aspergillus section Nigri. *Studies in Mycology* (50), 45-46.

Samson, R. A. and Reenen-Hoekstra, E. S. V. (1988). *Introduction to food-borne fungi Centraalbureau voor Schimmelcultures*. Delft, The netherlands: Baarn.

Sanguinetti, M., Posteraro, B., Pagano, L., Pagliari, G., Fianchi, L. and Mele, L. (2003). Comparison of real-time PCR, conventional PCR, and galactomannan antigen detection by enzyme-linked immunosorbent assay using bronchoalveolar lavage fluid samples from hematology patients for diagnosis of invasive pulmonary aspergillosis. *J Clin Microbiol, 41*(8), 3922-3925.

Satoh, H., Uozumi, T., Kiya, K., Ikawa, F., Kurisu, K. and Sumida, M. (1995). Invasive aspergilloma of the frontal base causing internal carotid artery occlusion. *Surg Neurol, 44*(5), 483-488.

Schaffner, A. (1992). *Host defense in aspergillosis*. Edinburg: Churchill Livingstone.

Scheidegger, K. A. and Payne., G. A. (2003). Unlocking the secrets behind secondary metabolism: A review of Aspergillus flavus from pathogenicity to functional genomics. *Toxicology-Toxin Reviews, 22*(2-3), 423-459.

Schneiderman, S., Farber, J. L. and Baserga, R. (1979). A simple method for decreasing the toxicity of polyethylene glycol in mammalian cell hybridization. *Somatic Cell Genet, 5*(2), 263-269.

Schuyler, M. (1998). The Th1/Th2 paradigm in allergic bronchopulmonary aspergillosis. *J Lab Clin Med, 131*(3), 194-196.

Segal, B. H. and Walsh, T. J. (2006). Current approaches to diagnosis and treatment of invasive aspergillosis. *Am J Respir Crit Care Med, 173*(7), 707-717.

Sharma OP and Chwogule R.(1998). Many faces of pulmonary aspergillosis. *Eur Respir J,* 12:705-715.

Sharon, J., Morrison, S. L. and Kabat, E. A. (1980). Formation of hybridoma clones in soft agarose: effect of pH and of medium. *Somatic Cell Genet, 6*(3), 435-441.

Singh, B. P., Banerjee, B. and Kurup, V. P. (2003). Aspergillus antigens associated with allergic bronchopulmonary aspergillosis. *Front Biosci, 8*, s102-109.

Stanker, L. H., Vanderlaan, M. and Juarez-Salinas, H. (1985). One-step purification of mouse monoclonal antibodies from ascites fluid by hydroxylapatite chromatography. *J Immunol Methods, 76*(1), 157-169.

Stanzani, M., Orciuolo, E., Lewis, R., Kontoyiannis, D. P., Martins, S. L. and St John, L. S. (2005). Aspergillus fumigatus suppresses the human cellular immune response via gliotoxin-mediated apoptosis of monocytes. *Blood, 105*(6), 2258-2265.

Stephan, S., Datta, K., Wang, E., Li, J., Brekken, R. A. and Parangi, S. (2004). Effect of rapamycin alone and in combination with antiangiogenesis therapy in an orthotopic model of human pancreatic cancer. *Clin Cancer Res, 10*(20), 6993-7000.

Stevens, D. A., Kan, V. L., Judson, M. A., Morrison, V. A., Dummer, S. and Denning, D. W. (2000). Practice guidelines for diseases caused by Aspergillus. Infectious Diseases Society of America. *Clin Infect Dis, 30*(4), 696-709.

Stewart, S. J. (2001). *Monoclonal Antibodies Production*: Boca Raton: CRC Press.

Sturtevant, J. and Latge, J. P. (1992). Participation of complement in the phagocytosis of the conidia of Aspergillus fumigatus by human polymorphonuclear cells. *J Infect Dis, 166*(3), 580-586.

Sulimenko, T. and Draber, P. (2004). A fast and simple dot-immunobinding assay for quantification of mouse immunoglobulins in hybridoma culture supernatants. *J Immunol Methods, 289*(1-2), 89-95.

Tarrand, J. J., Lichterfeld, M., Warraich, I., Luna, M., Han, X. Y. and May, G. S. (2003). Diagnosis of invasive septate mold infections. A correlation of microbiological culture and histologic or cytologic examination. *Am J Clin Pathol, 119*(6), 854-858.

Thom C, Church M.(1926). The Aspergilli. Baltimore: The Williams and Wilkins Company

Tronchin, G., J. P. , Bouchara, J. P., Latge´. and Chabasse., D. (1992). Application of a Lowicryl K4M embedding technique for analysis of fungal adhesins. *Mycol. Me´d*(3), 74–79.

Tsunawaki, S., Yoshida, L. S., Nishida, S., Kobayashi, T. and Shimoyama, T. (2004). Fungal metabolite gliotoxin inhibits assembly of the human respiratory burst NADPH oxidase. *Infect Immun, 72*(6), 3373-3382.

Underwood, P. A. and Bean, P. A. (1988). Hazards of the limiting-dilution method of cloning hybridomas. *J Immunol Methods, 107*(1), 119-128.

Verweij, P. E., Smedts, F., Poot, T., Bult, P., Hoogkamp-Korstanje, J. A. and Meis, J. F. (1996). Immunoperoxidase staining for identification of Aspergillus species in routinely processed tissue sections. *J Clin Pathol, 49*(10), 798-801.

Waldorf, A. R., Levitz, S. M. and Diamond, R. D. (1984). *In vivo* bronchoalveolar macrophage defense against Rhizopus oryzae and Aspergillus fumigatus. *J Infect Dis, 150*(5), 752-760.

Wall, R. and Kuehl, M. (1983). Biosynthesis and regulation of immunoglobulins. *Annu Rev Immunol, 1*, 393-422.

Warnock, D. W., Hajjeh, R. A. and Lasker, B. A. (2001). Epidemiology and Prevention of Invasive Aspergillosis. *Curr Infect Dis Rep, 3*(6), 507-516.

Washburn, R. G., Kennedy, D. W., Begley, M. G., Henderson, D. K. and Bennett, J. E. (1988). Chronic fungal sinusitis in apparently normal hosts. *Medicine (Baltimore), 67*(4), 231-247.

Westerwoudt, R. J., Naipal., A. M. and Harrisson, C. M. (1984). Improved fusion technique. II. Stability and purity of hybrid clones. *J Immunol Methods, 68*(1-2), 89-101.

Wewetzer, K. and Seilheimer, B. (1995). Establishment of a single-step hybridoma cloning protocol using an automated cell transfer system: comparison with limiting dilution. *J Immunol Methods, 179*(1), 71-76.

Wilson, E. V., De Magaldi, S. W. and Hearn, V. M. (1984). Preparative isoelectric focusing of immunologically reactive components of Aspergillus fumigatus mycelium. *J Gen Microbiol, 130*(4), 919-925.

Yan, Z. and Huang, J. (2000). Chromatographic behavior of mouse serum immunoglobulin G in protein G perfusion affinity chromatography. *J Chromatogr B Biomed Sci Appl, 738*(1), 149-154.

Yoo, J. H., Choi, J. H., Choi, S. M., Lee, D. G., Shin, W. S. and Min, W. S. (2005). Application of nucleic acid sequence-based amplification for diagnosis of and monitoring the clinical course of invasive aspergillosis in patients with hematologic diseases. *Clin Infect Dis, 40*(3), 392-398.

Zimmerman, R. L., Montone, K. T., Fogt, F. and Norris, A. H. (2000). Ultra fast identification of Aspergillus species in pulmonary cytology specimens by in situ hybridization. *Int J Mol Med, 5*(4), 427-429.

Zimmermann, U. (1986). Electrical breakdown, electropermeabilization and electrofusion. *Rev Physiol Biochem Pharmacol, 105*, 176-256.