

LLOYD M. STOOLMAN, M.D.
ASSOCIATE PROFESSOR OF PATHOLOGY
DEPARTMENT OF PATHOLOGY

ANNUAL DEPARTMENTAL REPORT
1 JULY 2000 - 30 JUNE 2001

I. CLINICAL ACTIVITIES:

- A. **Flow Cytometry Diagnostic Service** - interpretation of cell surface marker studies in the evaluation of hematologic disorders, primary and secondary immune deficiencies and autoimmune processes.
- B. **Autopsy Service**

II. TEACHING ACTIVITIES:

A. **Research mentor:**

1. **Nobuhiro Takeshita, M.D., post-doctoral fellow (April 1998-present) and Ronald Craig, PhD, Research Associate:** Dr. Takeshita is jointly supported by the L.M. Stoolman (Pathology) and A.E. Chang laboratories (Surgical Oncology) for work on T-cell trafficking during adoptive cellular immunotherapy for metastatic cancer. His research, conducted jointly with Dr. Craig, indicates that T-lymphoblasts grown from tumor-draining lymph node cells expressing binding sites for the adhesion receptor P-selectin (so-called Plig^{high} cells) are 10-100 fold more potent than cells derived from unfractionated populations against pulmonary and subcutaneous tumor implants. As few as 1×10^6 cultured Plig^{high} cells completely suppress pulmonary metastases generated by infusion of 3×10^5 murine sarcoma cells. The potency of the cultured Plig^{high} cells exceeds that reported for all previous forms of adoptive immunotherapy in the murine sarcoma model. Recent studies indicate that the Plig^{high} T-cells are responsible for tumor suppression in active immunotherapy as well. During the current year, these studies produced three manuscripts (1 in press, 1 submitted, 1 in preparation). In addition, Dr. Stoolman's R01 supporting this work was renewed and several projects based on the results were funded as part of a new R01 and P01 (Drs. L.M. Stoolman and A.E. Chang, co-investigators).
2. **Randall Knibbs, Ph.D., Research Scientist (January, 1994-present)** - Dr. Knibbs conducted toxicologic and functional studies on several novel inhibitors of the selectin family under an NIAID funded SBIR contract from Ligocyte, Inc. (project coordinated by Jon Nagy, PhD at Ligocyte and Lloyd Stoolman, MD at the University of Michigan). In addition, Dr. Knibbs and Melissa Allen in the laboratory developed adenoviral transfection vectors containing a gene-segment encoding murine L-selectin. This vector successfully conferred high levels of L-selectin expression in the HELA cell line. The vector will now be used to test the hypothesis that tumor-antigen pulsed, cultured murine dendritic cells expressing high levels of L-selectin will traffic more efficiently to lymph nodes and confer higher levels of tumor immunity in murine models. Finally, Dr. Knibbs participated in several collaborative projects investigating selectin ligand structure and function. These collaborative

studies resulted in six manuscripts (submitted or in preparation) during the current year.

3. **Graduate research assistants:** Mentored two Ph.D. and one MD/ Ph.D. candidates during laboratory rotations.
4. **Undergraduate research assistants:** Mentored two undergraduate students in the laboratory participating in work/study programs.

- C. **Director, General Pathology Laboratory Course for Dental Students (Pathology 631) and co-director, General Pathology Lecture Course (Pathology 630):** The 4th generation Virtual Microscope Pathology Laboratory Interactive Syllabus was deployed for the course (<http://141.214.6.12/cyberscope631/>). In addition, complete high resolution digital “maps” of selected microscopic slides were added to the site employing a new image server from the Bacus Laboratories, Inc. These “Websites” cover a larger area of the tissue sections (20-50%) than the single high resolution images currently in use. They can be zoomed and panned thus simulate the viewing experience through the microscope more accurately than previously possible. It is anticipated that this technology will initially supplement and, eventually, replace the glass slide sets currently employed in the classroom.
- D. **Co-director and lecturer, Hematology Sequence in Component II (Medical School 2nd year curriculum)-** Administered pathology component of sequence and co-directed course with Alvin Schmier, M.D. (Department of Internal Medicine and Pathology). The 4th generation of The Virtual Microscope-Hematopathology Interactive Syllabus was deployed for the course (<http://141.214.6.12/virtualheme99>). This site utilizes the image server and the general approach outlined above for the Dental Pathology Laboratory Website.
- E. **M1 Host Defense Sequence:** Lectured and developed CD-based courseware for lecture syllabus and case presentations.
- F. **Advanced Topics in Immunology:** Lecturer.
- G. **Pathology 581:** Co-director and lecturer.

III. RESEARCH ACTIVITIES:

ACTIVE SUPPORT (70% funded effort):

- A. **Principal Investigator- T Cell Trafficking in Adoptive Cellular Immunotherapy;** NIH, R01CA73059, 30% effort, \$180,000 (annual, direct); April 2001 -Mar 2005 (**RENEWED**).
- B. **Co-investigator on Project 2 and Co-director of the Immunology Core (with A.E. Chang, Surgical Oncology Division, University of Michigan)- Cellular Vaccines for Cancer Immunotherapy,** NIH P01CA59327, 15% effort, \$1,000,000 (annual, direct); June 2001-April 2006 (**NEW**).
- C. **Co-investigator (with A. E. Chang, Surgical Oncology Division, University of Michigan)- T-Cell Therapy of Human Renal Cell Cancer;** NIH R01CA69102, \$250,000 (annual, direct), 10% effort, (**NEW**, award pending).
- D. **Co-investigator and coordinator for Pathology Department section- “Rational Design of Adhesion Blocking Anti-Inflammatories”** (Jon O. Nagy, PI, Ligocyte Pharmaceuticals, Inc.); NIH, SBIR R43AI/GM43789, \$988,598 (annual, direct), 10% effort, June 1999-April 2001.**Co-investigator (with B. Richardson, Rheumatology Division, University of Michigan)- “Gender specific T-cell homing and**

autoimmunity”; NIH, R01AI42753, 10% effort, \$187,000 (annual, direct); Apr 1998-Mar 2003.

- E. **Co-investigator (with A. E. Chang, Surgical Oncology Division, University of Michigan)-“T-cell Activation for Cancer Immunotherapy”**; NIH R01CA82529, \$211,282 (annual, direct); 5% effort, Jul 1999-June 2004.
- F. **Co-investigator (with G. Kansas, Department of Microbiology/Immunology, Northwestern University)- “Leukocyte Recognition of P-selectin”**, American Cancer Society, \$120,000 (annual, direct), 5% effort, Jul 1999-June 2001.
- G. **Trainer on three funded pre-/post-doctoral training grants: Translational Immunology (J. Mule, PI); Surgery Oncology Research (A.E. Chang, PI) and Immunopathology (R. Miller, PI).**

IV. ADMINISTRATIVE ACTIVITIES:

- A. **Director of Research Flow Cytometry Laboratory and Co-Director of Clinical Flow Cytometry Laboratory-** managed the development of new software to interface clinical flow cytometry instruments with the Laboratory Information System (Cerner Millenium). Participated in the consolidation of Clinical Flow Cytometry and Hematology Laboratories. Managed the operation of the research flow cytometry instruments (provided access for departmental investigators with grant support for flow cytometry).
- B. **Co-Director, Hematology Sequence in Component II and General Pathology 580/630/631-** see educational activities.
- C. **Member, Medical School Curriculum Review Group**
- D. **Member, Learning Resources Center Oversight Committee**
- E. **Member, Medical School InfoTech Committee**
- F. **Member, Medical School and MD/PhD Admissions Committees**
- G. **Member, Pathology/Immunology Graduate Program Admissions Committee**
- H. **Participant, Retreat on Medical School Sequence II Content**
- I. **Member, Pathology Website Committee**

V. OTHER RELEVANT ACTIVITIES:

EDITORIAL ACTIVITIES:

- A. Journal of Clinical Investigation.
- B. Journal of Biological Chemistry.
- C. Journal of Laboratory Investigation.
- D. Journal of Experimental Medicine.
- E. American Journal of Pathology.
- F. Journal of Immunology (Associate Editor).

VI. PUBLICATIONS:

ARTICLES IN PEER REVIEWED PUBLICATIONS:

1. K. Tanigawa, K. Phillips, N. Takeshita, R.A. Craig , R.N. Knibbs, A.E. Chang and **L.M. Stoolman**. 2001. Journal of Immunology. Tumor-specific responses in lymph nodes draining murine sarcomas are concentrated in cells expressing P-selectin binding sites (in press).
2. Finn WG, Singleton TP, Schnitzer B, Ross CW, **L.M. Stoolman**. 2001. Human Pathology: Adhesion molecule expression in CD5 negative/CD10 negative chronic B-cell leukemias:

- comparison with non-Hodgkin's lymphomas and CD5 positive B-cell chronic lymphocytic leukemia. 32: 66 (2001).
3. **L.M. Stoolman**, R.A. Craig, M.J. Cameron, A.E. Chang. Ex-vivo expansion of human T-lymphocytes for adoptive immunotherapy: optimal growth conditions for expression of selectin ligands and attachment to vascular endothelium under shear. (submitted)
 4. M. Jutila, S. Kurk, L. Turk, R. Knibbs, **L.M. Stoolman**: L-selectin can serve as an E-selectin ligand on human T-cells. (submitted)
 5. A. Matsukawa, N.W. Lukacs, C.M. Hogaboam, R.N. Knibbs, D.C. Bullard, S.L. Kunkel and **L.M. Stoolman**: Mice Genetically Lacking Endothelial Selectins are Resistant to the Lethality in a Murine Model of Septic Peritonitis. (submitted)
 6. Wusi Maki, William G. Telford, Randall N. Knibbs, **Lloyd M. Stoolman** and Sam T. Hwang: CC chemokine receptor-6 (CCR6) co-localizes with CD18 and enhances adhesion to activated endothelial cells in CCR6-transduced Jurkat T cells. (submitted)
 7. Jeffrey L. Curtis, Joanne Sonstein, Ronald A. Craig, Jill Todt, Randall N. Knibbs, Timothy Polak, Daniel C. Bullard, and **Lloyd M. Stoolman**: Altered Lung Lymphocyte Recruitment in response to intratracheal particulate antigen challenge in mice lacking endothelial selectins is subset specific. (in preparation)
 8. N. W. Lukacs, R.N. Knibbs, A. Johns, **L.M. Stoolman**: E- and P-selectin molecules are involved in the inflammatory responses during allergen-induced airway hyperactivity. (in preparation)
 9. Daniel, Myers DVM, Dianna Farris LVT, Angela Hawley MS, Shirley Wroblewski BS, Amy Chapman BS, **Lloyd Stoolman MD**, Randy Knibbs PhD, Robert Strieter MD, Thomas Wakefield MD: Selectins and Interleukin-10 Influence Acute Thrombus Formation in a Mouse Model of Venous Thrombosis. (in preparation)

ABSTRACTS, BOOK REVIEWS, LETTERS TO THE EDITOR, MISCELLANEOUS UNREFEREED PUBLICATIONS:

1. **LM Stoolman**, Michael Lougee, Douglas Gibbs and Tom Peterson. 2000/2001. The Virtual Microscope- Interactive web-based syllabus for medical student (M2) Hematopathology laboratory. URL= <http://141.214.6.12/virtualheme99/>. The site incorporates high resolution (1900 X 1300 pixel) photomicrographs of blood smears, bone-marrow aspirates and lymph node sections in an interactive laboratory syllabus. Unique software allows user to pan across low-power images then magnify regions of interest. Questions (and answers) covering the pathophysiology, diagnosis and treatment of the hematologic malignancies are incorporated into the exercises. This "active" learning experience captures the essentials of the in-class laboratory exercises providing students with a flexible tool for preview and review. **1999 Computerworld-Smithsonian Award Finalist.**
2. **LM Stoolman**, Michael Lougee, Douglas Gibbs, Tom Peterson and Gerald Abrams. 2000/2001. The Virtual Microscope- Interactive web-based syllabus for General and Organ systems pathology for dental students (D2). URL= <http://141.214.6.12/cyberscope631/> This sites incorporates several hundred, high resolution (1900 X 1300 pixel) photographs of gross and microscopic specimens into an interactive laboratory syllabus. New this year are high resolution digital "maps" covering 20-50% of the microscopic section. These "webslides" emulate glass slides more precisely than single photographs. The features are as described above. **1999 Computerworld-Smithsonian Award Finalist.**

3. **LM Stoolman.** 2000/2001. Hematopathology Unknown Exercises. Interactive, CD-based excercises for medical student (M2) Hematopathology laboratory. CD-based publication used in M2-Heme sequence.
4. **LM Stoolman.** 2000/2001. Leukocyte Pathophysiology and Leukocyte Trafficking. Interactive, CD-based syllabus and exercises including video clips and animations. CD-based publication used in Pathology 581 (Graduate Course) and Host Defense Sequence (M1 sequence).

