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ABSTRACT

IMMUNOLOGICAL INSTRUCTIONAL UNIT

By

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Lacking the academic motivation instilled by final examinations and noting a waning level of senior interest, H. H. Dow's Advanced Biology course needed a year ending "academic fix". A newly designed, activity based, instructional unit in human immunology enhances student interest, involvement, and academic achievement while coincidentally encouraging review and application of previously encountered core biological concepts.

A pedagogically sound sequencing of practical laboratory exercises, varied immunological readings, and thought provoking simulations invite total student involvement. Improved participation and strong, cohesive subject content facilitate student comprehension and elevate the academic value of the final weeks of school.

Statistically significant increases in student post test performances compared to earlier pretest results validate unit effectiveness. Favorable student and teacher observations regarding learner perceptions and behaviors confirm the value of the instructional unit. Finally, test item analysis indicates instructional strengths and weaknesses completing unit assessment and promoting future improvements.

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This work is dedicated to my mom, dad, and wife.
Patricia and Harold, thank you for teaching
your children how to work hard, play hard,
and always love one another.

Anne Marie, thank you for your timely
pep talks and enduring love.

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INTRODUCTION

Immunity refers to the anatomical and physiological components of an organism serving to protect it against foreign environmental agents. These foreign agents include microbial pathogens and their chemical by-products, drugs, chemicals, foods, and pollen. The components of immunity and their actions are classified as innate or acquired.

The components of innate immunity are generally present and functional at birth and include: the skin, mucous membranes, natural killer cells, neutrophils, lysozyme, interferons, and the maintenance of regional acidic pH.

The activities of innate immunity are many and varied. Interferons are typically antiviral in nature. Lysozyme catalyzes the destruction of bacterial cell walls. Neutrophils are utility phagocytes ingesting and digesting different harmful chemical and cellular complexes. Acidic pH proves unfavorable for the growth of many potential pathogens. Natural killer cells attack newly formed cancer cells.

Many innate mechanisms act as physical or chemical barriers blocking the penetration and propagation of the infectious agent. Innate actions often negate threatening foreign agents preventing serious disruption of organism homeostasis.

Unlike innate immunity, the activation of the immune response (acquired immunity) requires contact with the invading entity. The response of B and T lymphocytes serve as the "heart and soul" of this system. These cellular workhorses are capable of distinguishing self, one's natural

cells and molecules, from nonself and mounting appropriate attacks against non-self chemical and cellular agents.

Specificity and memory are fundamental features of acquired immune responses. Specificity lies in the ability of B and T lymphocyte receptor molecules to recognize antigenic regions called epitopes. Foreign chemicals capable of triggering an acquired immune response are known as antigens. Epitopes are chemical subsets of antigens. Each unique B and T cell clone is marked by its characteristic receptor molecule. Ultimately, specific clones are activated by specific epitopes or antigens.

Memory, another trademark of acquired immunity, is manifested in the rapid B and/or T cell response observed when exposure to the specific foreign agent is a repeat experience. This repeat immune response is termed the second set response and is triggered by the activation of memory B and T cells. It is notably faster and more intense than a first time immune response.

Acquired immune responses are further divided into humoral and cell-mediated responses. Humoral immunity is the result of B lymphocyte activation with the subsequent production of immunoglobulins or antibodies. Cellular immunity occurs as T lymphocytes are called into immune combat.

In humoral immunity, antigens (epitopes) trigger B lymphocyte signal transduction and resultant gene activation. Gene activation leads to rapid mitosis. This is followed by cellular differentiation and the formation of large numbers of plasma cells. Plasma cells produce copious amounts of antibody specific for the initial antigen.

Cellular immunity occurs as specific T lymphocyte clones are activated when any of a number of antigen presenting cells (APC's), carrying appropriate antigen, contact them. Activated T lymphocytes undergo intense mitosis and cellular differentiation leading to several active forms of T lymphocytes, most notably helper and cytoxic T cells.

Helper T lymphocytes, the unfortunate preferred host of HIV, coordinate the entire acquired immune response. Helper T cells regulate the activities of other forms of T lymphocytes and strongly influence the ability of B lymphocytes to form antibodies.

Cytoxic T cells locate foreign and irregular cells and kill them with secretions of cytotoxins. Common cellular targets of cytoxic T cells are virally infected cells and cancer cells.

In order for acquired immunity to function properly, an array of molecules are essential. Specific receptor molecules mark the identity of immune competent cells. Major histocompatibility complexes of type I and II (MHC I and MHC II) are essential to distinguishing self from nonself and coordinating the interactions of T lymphocytes and APC's. Numerous molecular messengers known as cytokines allow communication and coordination between the various immune cell types. Antibodies and cytotoxins are the ultimate chemical products of the acquired immune response. Ultimately, immune competence hinges on the ability to distinguish self from nonself and is recognized when organisms enjoy good health (Benjamini and Sunshine, 1996).

The science of immunology is an applied biological discipline drawing from many core biological concepts.

Comprehending the workings of the immune response, related research, and clinical applications requires a sound background in molecular, cell, and organismal biology. The study of immunology summons the review and application of many Advanced Biology course concepts and therefore serves as an ideal capstone learning experience.

With the advent of the Germ Theory, the growth of immunology became imminent. The dawning of the AIDS plague commanded a vigorous, scientific effort. As a result, the applied biological science of immunology has been upgraded and improved almost to the point of reinvention. In addition to HIV and AIDS, studies in tissue transplantation, allergies, vaccines, and autoimmune disease amplify immunology's relevance, even for high school seniors!

Statement of Problem and Rationale for Study

As graduation approaches, seniors in high school generally experience a natural emotional state known as separation anxiety. The manifestations of this condition include; increased absence and tardiness, inadequate preparation, reduced academic expectations, and an accelerated disrespect for authority. These behaviors are often clumped under the heading of "senioritis" (Mitchell, 1995).

Following suit, Advanced Biology seniors demonstrate a notable decline in academic interest and effort. As graduation approaches the level of academic malaise increases. A growing number of late and incomplete assignments, reduced attention spans, increased diversionary behaviors, and negative verbal and nonverbal expressions are

further testimonies that "the plague" has arrived. Communicable in nature, juniors soon contract senior attitudes and classroom productivity and morale suffer.

Additionally, many Advanced Biology students experience academic fatigue as the school year comes to a close. Our course is academically demanding and many of our students pursue additional honors course work while balancing extensive extracurricular and community involvements. Simply put, many of our students are physically and mentally drained.

Several years ago, in an effort to offset senior apathy and its negative side effects, a series of observational microbial laboratory exercises were introduced. Student responsibilities focused on recognizing species-specific anatomical features of selected algae, protozoa, and bacteria. The investigation concluded with short readings featuring the behaviors and general ecological roles of Protista and Monerans.

Academic requirements were simplified and streamlined consisting of labeled sketches and written responses to a few study guide questions. Successful completion of the investigation did not require higher level thinking and analysis. Homework was reduced.

Initially, student involvement and participation improved. Grades rebounded and class time was productive. Our response to senioritis seemed effective. However, three years ago, a notable decline in student performance began. Disturbing, nonproductive student behaviors became increasingly prevalent. Non-biology related conversations and thoughtless copying of laboratory sketches were

particularly upsetting to this instructor.

> "Its hard for faculty not to take senioritis personally. Being letdown by the seniors prevents clear thinking necessary for innovative solutions" (Mitchell, 1995).

Reasons for such a decline may lie in recent curriculum changes. Four years ago our school system brought the ninth grade into high school and established middle schools. Up to this point in time, the vast majority of Advanced Biology students had taken their general biology in a junior high setting with less sophisticated laboratory experiences and expectations.

Accompanying these changes was a district-wide effort to enhance the laboratory experiences offered in first year high school biology. Teachers instituted several microbial lab exercises. As a result, more recent Advanced Biology students may view our year ending labs as repeat experiences. Additionally, our first year course is more process-oriented. Lab activities are generally more dynamic. Students are experiencing more involved set-ups and manipulations coming closer to approximating true scientific investigation. Students have gradually developed heightened expectations of what they deem a meaningful lab investigation.

Ultimately, our once satisfactory treatment for senioritis had become ineffective and obsolete. Advanced Biology's final weeks were in need of a curricular overhaul.

An additional personal concern also called for an instructional change. Increased intrusions on instructional time have caused a strain on the Advanced Biology curriculum. As biological insights continue to expand, our time to relate

these exciting advancements to previously mastered core concepts has diminished. Consequently, devoting our final school weeks to microbial observation labs became unacceptable. Students would be better served by an academically strong series of relevant learning experiences.

As a result of research experiences at Michigan State University, I gained interest and background knowledge in immunology and related topics. Under the tutelage and guidance of several MSU professors, laboratory experiences in Ouchterlony assays, radial immunodiffusion assays, serum electrophoresis, and the induction of humoral immunity in Periplaneta offered new curricular opportunities (Rheins and Karp, 1982). Additional readings in immunology texts and periodicals led to the generation of instructional resources.

Through this research it became readily apparent that a properly constructed instructional unit in immunology had the potential to squelch senioritis and boost the academic value of our final school weeks. It was time to launch an attack on senioritis and a healthy dose of immunology seemed most appropriate.

Pertinent Demographic and Background Information

Dow High School students in this study hail from Midland County, population 80,669 with most coming from the City of Midland, subset population of 40,210. Ninety-six percent of Midland residents are Caucasian while Asians and Pacific Islanders are the next most populous groups followed by African-Americans, Hispanics, and Native-Americans. These county-wide percentages are in contrast to our student

subject group of which twenty-four percent are minorities, primarily of Asian heritage.

The four largest employers in the County of Midland are two large production-based chemical firms, a hospital, and the Midland Public School System. Recent Michigan Employment Service Agency statistics indicate that no other region in the country has a greater per capita population of chemical engineers, chemists, and metallurgists. Per capita income in the county exceeds state and federal levels by over four thousand dollars. Although the County contains rural pockets of poverty, the overall socioeconomic status of Midland County appears healthy. The majority of subjects come from middle to upper-middle income households. These factors are significant indicators of the area's high priority on education.

The 42 subjects included 31 seniors, 10 juniors, and 1 sophomore. The gender distribution was fairly even with 23 females and 19 males. As mentioned earlier, twenty-four percent were minorities, primarily of Asian heritage. The average student grade point was 3.82 (on a weighted scale of 4.6). The lowest g.p.a. was 2.9 while the highest was 4.55. All students indicated they were college bound. Over half will select a science-math based collegiate curriculum.

Although individual levels of effort, motivation,and academic abilities characterize the subjects, it can be safely assumed that the average subject is highly motivated, academically strong, and willing to put forth a sincere effort. Students who elect (generally anywhere from one-third to one-half of the class) to take the Advanced Placement test in Biology typically fare very well. Annual

Advanced Placement performance averages fluctuate, but are always above 4.5 with 5.0 being the highest possible score.

Advanced Biology is an accelerated elective course that meets two hours daily. The time allotment allows for numerous laboratory explorations and various enrichment activities beyond core lecture-discussion presentations. Solid financial support and a progressive in-house administration encourage the evolution of course content and related learning experiences.

Review and introductory content is presented at the high school level with concept expansion to the collegiate level. Topics of emphasis are ecology, basic cell anatomy and physiology, basic biochemistry, human tissues, human anatomy and physiology, fetal pig dissection, genetics, cell respiration and photosynthesis, general microbiology, and now, immunology. Historically, returning college students speak highly of the laboratory skills and content knowledge accrued during their Advanced Biology tenure.

Content and Philosophy of The Immune Instructional Unit

A review of educational programs successfully countering the negative aspects of senioritis revealed the following commonalities: higher academic expectations, relevant learning experiences, a wide variety of learning activities, a sense of student empowerment, and a system of rewards (Sachs 1998; Haffey, 1995; Mitchell, 1995). A sincere attempt was made to incorporate these features into the immunology instructional unit.

Additional factors governing instructional unit composition included reduced homework, conceptualization from

general to specific, mastery of anatomy as a precursor to understanding physiology, and spiral learning characterized by the progressive compilation of relatively simple concepts yielding complex understandings.

Learning activities, with the exceptions of labs and a few readings, were typically held to a maximum of twenty minutes. Each learning activity ended with students completing a brief review and assessment exercise intended to validate their mastery of the activity's goals and motivate their progress toward the next activity.

The study of immunology was divided into five areas: general anatomy and physiology, cells of immunology, key molecules of immunology, total immune response, and related immune topics (HIV/AIDS, allergies, vaccines, transplantation, and autoimmune diseases). The learning activities pertaining to each of these five topics were organized into a corresponding learning packet. The contents of the five learning packets and the relative class time given to each activity is shown in Table 1 on pages 12 and 13.

Many of the learning activities within the packets were developed at Michigan State University during a summer research course. Each packet contained a collection of various spiral learning activities and labs designed to facilitate mastery of the immune instructional unit's objectives (Appendix A2).

Although each packet was unique, common packet elements include: teacher-generated materials (readings, diagrams, study guides, activities), lab exercises, periodical and text readings, diagrams and pictures from various published resources, and classroom and computer simulations.

A sample of the Introduction to Immunology learning packet can be seen in Appendix B1. Note that labs are taken out of their packets for purposes of quick referencing and ease of observation. Certain copyrighted, colored diagrams available to students are missing from the Appendix version of the packet.

Table 1-Calendar of Instructional Activities

May 9	-Pretest
May 9-10	Packet One -Introduction to Immunology Learning Packet
May 10-11	Packet One -Nonspecific Immunity-A teacher directed lecture-discussion Packet Two-Cells of the Immune System Learning Packet
May 12	Packet Two-Laboratory Exercise-Examining Cells of the Immune System Packet Two-Laboratory-Ouchterlony
May 15	Packet Two-Ouchterlony Lab Analysis Packet Three-Antibody-Antigen Interaction Packet Three-Key Molecules of the Immune Response
May 16	Packet Three-Clonal Selection and the Activation of B Lymphocytes Packet Three-Antibody Structure and Function Presentation- Packet Three-Laboratory-Detection of HCG
May 18	Packet Three-Reading Exercise: (homework) "How the Immune system Develops" Packet Three-Small Group Discussion-Ig Genes Packet Three-Laboratory Exericse-Radial Immunodiffusion
May 19	Packet Three-Reading: "The Secret of Our Success" Arousing the Immune System's Fury Packet Four-Applied Immune Responses to: Pneumococci, Influenza, and Leishmania.

Table 1 - Calendar of Instructional Activities (continued)

May 22	Packet Four-T-Cell Activation of B Lymphocytes Packet Four-Radial Immunodiffusion Lab Analysis Packet Four-Pre-Lab for Virtual Diagnosis of SLE Packet Four-Laboratory-Computer Simulated Detection of SLE
May 23	Packet Four-Reading: "The Killers That Save Us" Arousing Fury of the Immune System Packet Four-Laboratory-Immunoelectrophoresis
May 24	Packet Four-Immunoelectrophoresis Lab Analysis Packet Five-Understanding Autoimmune Disorders Packet Five-The Life cycle of HIV
May 25	Packet Five-Laboratory: The Western Blot Detection of gp-120; a conclusive test for HIV.
May 26	Packet Five-Results Interpretation
May 30	Packet Five-Immune Considerations in Transplantation Packet Five-Immune Test Objectives and Review
May 31	-Final Test

IMPLEMENTATION OF THE IMMUNOLOGY INSTRUCTIONAL UNIT

Students were given a UCRIHS approved permission letter (Appendix A1) explaining general unit content and requesting the right to use their grades and learning products as data for unit assessment. All students and parents consented.

Prior to instruction, students received an outline of the immunology unit's activities and objectives (Appendix A2). A brief explanation of goals, objectives, and instructional philosophy followed. A major point of emphasis underscored the inverse relationship between in-class student focus and homework load. Students were also told that the entire immunology instructional unit was designed to motivate and excite their participation.

A pretest followed (Appendix E1). This test consisted of twenty multiple choice questions covering a variety of immunological concepts, eight immune system anatomical labels, eight corresponding brief response questions regarding organ function, ten fill-in-the blank vocabulary items and three short response questions focusing on general immune physiology.

These same items would later become a subset of a larger, more complete post test (Appendix E2) that would serve two purposes: 1) providing data for evaluating instructional unit effectiveness (a before and after profile); and 2) serving as criteria for individual student assessment.

Students were encouraged to perform to the best of their ability, treating every question seriously by responding with any pertinent knowledge or understanding they possessed.

Uneducated guessing was discouraged. Students were asked to be realistic in their expectations and were aware of the fact that this test would be used as a baseline for post test performance.

Assessing the Immunology Unit's Instructional Strength

The assessment of the instructional unit's effectiveness was primarily based upon student outcomes. Comparative subject performances on pretest and post tests were statistically analyzed in a one-tailed test at the .05 level of significance. Due to the number of subjects a z-test, rather than a t-test, was used with a critical score above 1.645 resulting in rejection of the null hypothesis.

Student learning products provided the most effective measuring tool for determining instructional unit worth. Essay and short answer responses to lab, post test, and article questions were highly scrutinized and most indicative of student growth.

A thorough item analysis comparing pretest and post test changes provided excellent insights into strengths and weaknesses of the unit. Finally, what could be more revealing than observations of student behaviors while under the influence of the instructional unit?

Introductory Learning Activities

Students began their investigation into immunology by performing the activities in the *Introduction to Immunology* learning packet (Appendix B1). Each student received an individual packet and verbal instructions.

Key scientific breakthroughs in immunology accompanied

by dates and the investigators opened the learning packet. These materials invited student participation while generating an overview of immunological issues, advances, and terminology.

As students worked individually on initial exercises, the teacher conferred with them as questions arose. Occasionally, a student query led to a teacher response deemed essential to all learners and packet work was interrupted, allowing total class attention. Valuable learning occurred during these ad hoc, student-driven sessions. Students shared unique personal experiences and generated a variety of tangential questions stimulating practical discussions in immunology.

Students gained a sense of empowerment as they exercised their freedom to steer productive discussions. Similar bouts of open discussion were a part of all learning packets.

Following the historical summary of immunological breakthroughs, students were introduced to Edward Jenner's development of a smallpox vaccine and a closeup view of micrographs highlighting twenty-nine of man's most formidable pathogens and parasites (Hall, 1996). These colorized micrographs are visually intriguing and intended to arouse student curiosity. Students were invited to ponder each pathogen's identity by attempting to match corresponding micrographs and names. An unkeyed list of names was included. Later, a pathogen key allowed students to return to the micrographs for a second analysis. Coincidentally, students familiarized themselves with notorious pathogens of the immune system while intrinsically recognizing immunity's crucial role in organismal survival.

Students labeled a human figure highlighting basic immune organs (Nossel, 1993). Appropriate labels were included later in the packet allowing students to correct their work. Students investigated key functions of the labeled organs in a concise reading. A fill-in-the-blank, short answer activity allowed students to recall and apply their introductory level immune organ anatomy and function knowledge (Appendix B1).

The anatomy of the immune system was further explored utilizing the The Human Anatomy Coloring Book (Kapit and Elson, 1993). Each student examined the gross and micro anatomy of the thymus, spleen, lymph nodes, tonsils, and bone marrow, as well as, lymphatic circulation. In addition to beautifully detailed sketches, each diagram is accompanied by a concise and thorough explanation of physiology. A series of teacher generated questions sparked a classroom discussion emphasizing organ functions. A brief free-write session followed in which students demonstrated their comprehension of the relative anatomy and physiology.

Three fifteen minute lecture-discussion segments highlighting the components and functions of the non-specific immune players concluded our introduction to the immune system. Items such as skin, mucosal barriers, body acids, complement, interferon, lysozyme, natural killer cells, and normal flora were discussed.

Cells of The Immune System

A second teacher generated instructional packet entitled, *Cells of The Immune System*, focused upon the B and T lymphocytic warriors of the immune system

(Appendix B2). Students analyzed the development, structural features, and general functions of these cells. Johns Hopkins Atlas of Functional Human Anatomy (Schlossberg, 1986) served as an excellent visual resource documenting leukocyte development and guiding laboratory identification.

Following a discussion of the general disease fighting activities of B and T lymphocytes, students examined a flow chart highlighting the histological links and functions of leukocytes and their relatives. A colorful data table featuring micrographs of cells and their functions allowed students a third look at key immune system cells. Students wrapped up their pictorial cell observations with micrographic views of an activated T lymphocyte and macrophage (Campbell, 1996). The utilization of a variety of resources provided acceptable repeat learning experiences essential to fostering concept mastery (Elkins,1997).

Examining Cells of the Immune System

In this observational laboratory (Appendix D1), students familiarized themselves with lymphocytes and other white blood cells. Students used prepared human blood slides and counted 50 individual leukocytes after reviewing the major categories. A primary student objective was the proper identification of each of the fifty white cells followed by a differential count of the leukocyte forms.

Special recognition (highly prized and very rare extra credit points) was given to discoverers of basophils. Upon making an identification, students were encouraged to recite functions.

The exercise concluded with an examination of blood

slides from acute and chronic lymphocytic leukemia patients. Specific questions regarding the comparative appearances of leukemic and normal blood slides encouraged the application of immune concepts. Students also reviewed concepts in circulation.

Students documented their experience and concept mastery by completing a laboratory report form (Appendix D1). Correct laboratory responses included a summation of the functions of all mature common stem cell derivatives, except erythrocytes and platelets.

The Ouchterlony Laboratory

Next, students began the Ouchterlony laboratory investigation (Appendix D2). Antibody-antigen interaction is responsible for a large part of immune competence. This interaction can be characterized by precipitation or the formation of insoluble complexes. Optimal complex formation occurs when antibody and antigen molecules are roughly equal in concentration.

Utilizing the Ouchterlony Test, students learned to identify a specific antigen or antibody in a sample when one or the other of these complementary partners was supplied.

Advanced applications of Ouchterlony's technique allowed the identification of multiple antigen-antibody systems as indicated by a layering of precipitin bands. Further advanced applications revealed cross-reacting antigenic determinants as illustrated in the Evaluation section (Figure 1).

Students were responsible for generating accurate Ouchterlony plates and explaining the molecular events

leading to various precipitin band formations. Plate sketches and responses to analysis questions completed lab requirements (Appendix D3).

Key Molecules of the Immune System

Following a brief pictorial presentation of antibody-antigen interactions, students began the *Key Molecules of the Immune System* learning packet (Appendix B3). Again, students received their own packets and worked individually. while the instructor interacted with pupils on a per need basis. Intermittent class discussion was commonplace.

The immune molecules emphasized included: CD-4, CD-8, MHC I and MHC II, interleukins, cytoxins and transducer molecules. Special attention was given to the cellular sources, targets, and ultimate functions of the molecules. Students completed a written review guide solidifying their perceptions of immune molecules. A general overview of molecular functions was emphasized.

A highly detailed table describing the interactions of the various cytokines and their cellular targets provided further practice, review, and additional insights (Goldsby et al., 2000). This table highlighted the complex multiple functions and interactions of various cytokines, a reality sometime overlooked in first level presentations.

The structure and function of immunoglobulins was given special emphasis in this learning packet. Students examined a diagrammatic representation of antibody-antigen interaction. The concepts and applications of precipitation, agglutination, complement activation, and neutralization were discussed and practical examples of each scenario were given by the instructor. The role of macrophages in final clean-up

provided a review lesson in lysosomes.

Students examined a labeled two dimensional immunoglobulin sketch and read a concise informational paragraph on antibody structure. Five volunteers were given ninety seconds to draw their interpretation of the general form of an antibody; five additional volunteers were assigned to the original sketches and given thirty seconds to add their improvements. With class input, a two-dimensional concept of the antibody was developed. This activity extended to a discussion of the five classes of immunoglobulin molecules.

Students were asked why mother and child Rh incompatibilities were historically more of a health threat than ABO incompatibilities. This led to a practical differentiation of IgG's and IgM's. Several students raised questions regarding allergies and IgE's, leading to a discussion involving various aspects of the allergic response.

A three page reading summarizing antibody structure and function was assigned as homework. A short quiz on antibodies would start the following class meeting (Appendix E3).

Clonal Selection of B Lymphocytes

A critical diagram (Appendix B4) depicting clonal selection and the activation of B-lymphocytes provided a framework for a lecture-discussion emphasizing key immune concepts including self recognition. The discussion followed with a second diagram illustrating the antigenic signal transduction mechanism leading to B-cell proliferation and

the ultimate differentiation to a plasma cell with antibody making ability. Concepts in gene activation, membrane structure, and cell physiology were revisited with a special emphasis placed on the phospholipase C, the second messenger diacylgycerol, calcium, protein kinases and transcription factors. Students were encouraged to jot notes on their diagram and strive for the general overview of cell transduction (Appendix B5).

Monoclonal Antibody Production

Students utilized their text, Genentech's Access Excellence, (http://www.accessexcellence.org/wn/index.html), and associated diagrams (Appendix B6) to gain an understanding of monoclonal antibody production.

Teacher-generated questions regarding myeloma cells, mouse (nonhuman mammalian cells) cells, and hybridomas sparked a discussion leading to a summary of the steps taken to produce "designer" antibodies. Students were encouraged to suggest potential uses for monoclonal antibodies prior to a prelab explanation of the use of monoclonal anti-HCG in pregnancy testing.

Use of Monoclonal anti-HCG in Pregnancy Testing

Utilizing human chorionic gonadotropin (HCG) obtained from Sigma Chemical and kit materials developed by Wampole Laboratories, students ran an antigen-antibody test for the presence of the hormone of pregnancy, HCG, in mock human urine samples. The precise student procedure and experimental rationale is found in Appendix D4.

Upon completing the assay, students were responsible for

communicating their results to the instructor. The following class meeting students were presented with this quiz question, "Explain the immunochemical events responsible for a positive pregnancy test."

Scientific American Reading: How The Immune System Develops (Cooper and Weissman, 1993)

This September 1993 article describes the process of B and T lymphocyte clone establishment. Students read this article at home and were asked to document five new insights garnered from the article. These were shared at the beginning of our next class.

Readings: The Genetics of Antibody Determination

The text (Campbell, 1996), provided excellent diagrams and explanation of the genetics of antibody determination. The nature of the constant, variable, and junction regions of the genes were clarified. Gene plasticity and rearrangement was clearly illustrated. Gene rearrangement was tied to B-cell differentiation.

Following a short discussion period, students were presented with an interview of Susumu Tonegawa, 1987 Nobel Laureate; who solved the riddle of how a few genes can code for literally billions of different antibodies. Stephen Hall, writing for the Howard Hughes Medical Institute, conducted the lively interview. Tonegawa reviewed his research, thought processes, and utter excitement. Students had an opportunity to feel the elation of scientific discovery.

The article continued to describe the findings of

Mark Davis in delineating the genetics of T-cell receptor determination. Although the story turns out to be somewhat similar to the work of Tonegawa and B-cells, the discovery of a gamma T-cell is puzzling to immunologists. Many students expressed their satisfaction with this enlightening and enjoyable article. Students wrote a one paragraph summary explaining the genetics of antibody determination.

RID-Radial Immunodiffusion Laboratory (Edvotek, 1998)

Radial immunodiffusion is a technique used to determine the concentration of a given antigen or antibody in a sample. This technique can be used clinically to detect patient levels of a specific blood protein.

Students were given the task of determining the concentration of an antigen in a serum sample. The basis for determining this unknown concentration was the generation of a best line standard curve derived from laboratory measured antibody-antigen reactions with known concentrations of the antigen in question.

Edvotek Biotechnological provided the general procedure which was adapted to meet classroom situations. The adapted procedure is outlined below.

Students prepared an agarose gel plate and cut wells from the plate using a small plastic straw. A central well was surrounded by five equidistant outer wells. The peripheral wells received serial dilutions of known antigen concentrations while the central well received the sample containing an unknown amount of antigen. Antibody complimentary to the antigen of concern was incorporated into the liquid agarose just prior to solidification (antibody is

temperature sensitive, agarose must cool to 55 degrees Celsius) and evenly dispersed throughout the hardened agarose gel. As antigen diffuses from the various wells, rings of precipitation form radially. A period of 48 hours incubation at 37 degrees Celsius is required for optimal precipitin ring formation.

Students measured the diameter of each precipitin ring to the nearest 0.2 mm. By plotting the known antigen concentrations on the X-axis versus their respective precipitin ring diameters squared on the Y-axis, a best fit line was derived that served as a standard curve for determining the concentration of unknown antigen.

Students were responsible for data analysis, standard curve formulation, estimating the concentration of unknown antigen and responding to four laboratory analysis questions (Appendix D3).

Specific Immune Responses to Three Pathogens

Tailored after explanations and diagrams contained in the article; Infectious Diseases and the Immune System (Paul 1993), a classroom discussion focused on the specific B-cell, T-cell, and macrophage activities associated with the immune response to influenza, pneumococci, and Leishmania. These diagram-guided explanations encouraged students to apply their recently acquired immune cell and molecular insights to specific scenarios of total immune response. In essence, these scenarios allowed us to "put it all together", with respect to the immune response.

Students were alerted to the fact that any or all of these disease fighting scenarios could appear on their final

test. This provoked a few additional questions.

Helper T Activation of B Cells

Realizing that the HIV virus can completely shut down the immune response by selectively infecting helper T cells, students were presented with the dilemma of antibody titer declines characteristic of AIDS patients. Several students recalled that activated helper T cells are needed to promote antibody production by B lymphocytes. Utilizing a flow chart from Human Anatomy and Physiology (Marieb, 1999), students designed a sketch indicating the role of helper T's in activating B cell mitosis, differentiation, and subsequent antibody formation.

Howard Hughes Medical Institute ELISA Simulation: Detecting Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is an excellent example of an autoimmune disorder. The diagnosis of this disorder employs monoclonal antibody technology and ELISA, enzyme linked immunosorbant assay, two common and powerful procedures in current immunological diagnoses.

Prior to beginning the computer lab simulation (HHMI, http://www.biointeractive.org/) diagnosing SLE, students read a brief background summary clarifying aspects of the disease. A question and answer session followed. Students also used scissors and puzzle-shaped, paper representatives of antibodies, antigen, enzyme, and substrate to fashion a working model of an enzyme-linked antibody (Baker, Moore, 1996).

With an entry level background in SLE and ELISA,

students logged on to the Howard Hughes Medical Institute's Virtual Laboratory site where they carried out the laboratory diagnosis of SLE. Background information explained the laboratory quest to identify anti-DNA antibodies characterizing the serum of afflicted individuals. A thorough laboratory protocol and a detailed virtual laboratory procedure gave students the ability to execute a complete simulated analysis for anti-DNA antibodies.

Hasty or improper technique led to erroneous results which were pointed out by the computer program only after the simulation was completed. This feature, once discovered, encouraged students to take their time and focus on each step of the protocol. Laboratory errors were identified and the students began the analysis anew.

It should be mentioned that this animated lab simulation offered excellent graphics and a realistic experience. Following satisfactory execution of the laboratory, students showed their spot-plate results to the instructor and responded to four questions probing comprehension of laboratory protocol and outcomes (Appendix D3).

HHMI Reading: The Killers That Save Us (Hall, 1998)

This fascinating article introduced students to an HIV positive patient who was receiving his own CD8 T cells as therapy against HIV infected cells. Following their selective isolation, the HIV-sensitive killer T cells were grown in tissue culture and periodically infused into the patient. The article provided students with a "user-friendly" explanation of the general process involved in this new form of therapy.

Students were exposed to current applications and technologies of T-cell selection, specifically their use against Cytomegalovirus and residual immune competent cells in recovering bone marrow recipients. Students were required to write a one page summary of their impressions of the article's content.

Laboratory Exercise in Immunoelectrophoresis (Edvotek, 1998).

Immunoelectrophoresis is a widely used clinical and research technique teaming the separation capabilities of electrophoresis and the specificity of antibody-antigen interactions to identify proteins.

Immunoelectrophoresis has diagnostic capabilities when examining a variety of body fluids such as serum, urine, pleural fluid, cerebrospinal fluid, etc. Research applications of immunoelectrophoresis include monitoring the purity of antigen or antibody preparations and detecting soluble antigens from various microbial, plant, or animal extracts (Edvotek, 1998).

Following the electrophoretic separation of a mixture of proteins, students expose the proteins (antigens) to diffusing antibodies of known identity. Following a 24 hour incubation period (48 hours provided better resolution), the interpretation of precipitin bands led to final protein identification.

Student responsibilities included a verbal explanation of final gel precipitin results, a sketch of their precipitin patterns, and response to five lab analysis questions (Appendix D3).

Autoimmunity, HIV, and Introduction to Western Blotting

A class discussion regarding autoimmune diseases and their immunological causes followed a brief reading from Human Anatomy and Physiology (Marieb, 1998). Diseases emphasized were diabetes mellitus, multiple sclerosis, rheumatoid arthritis, and previously investigated SLE.

Next, students were paired and asked to review the text's (Campbell,1996) presentation of the infectious cycle of HIV, and make a sketch illustrating the chronological steps of viral propagation. Teacher directed discussion finalized a detailed life cycle of HIV with special emphasis placed upon gp 120, CD4, uncoating, reverse transcriptase, cDNA, provirus formation, and budding.

Laboratory Exercise-Detection of HIV via Western Blot (Edvotek, 1998).

Students combined electrophoresis with a technique known as Western Blotting to simulate the procedure used to determine the presence of glycoprotein 120 (specific to the human immunodeficiency virus) in serum.

In screening the blood supply, individual sera are tested utilizing enzyme linked immunosorbent assays or ELISA. This test involves the use of antibodies against immuno-globulin G specific for HIV. It serves as a first round or preliminary test due to potential cross-reactivity. The definitive test for the presence of HIV is Western Blot screening for the HIV envelope protein GP-120 (Edvotek 1998).

In this laboratory exercise, techniques were authentic but materials of a nonhuman, non-HIV nature, were used to simulate actual situations. Hypothetical samples of

lymphocyte cultures exposed to serum from three ELISA diagnosed "HIV positive individuals" were separated and the growth media of each separate culture was collected and concentrated. Students began with the hypothetical growth media concentrates. If these three individuals truly were HIV positive, the viral envelope protein gp-120 would be readily identifiable in the growth media through Western Blotting methods.

Prior to electrophoresis, each sample has its proteins denatured and dissociated utilizing sodium dodecylsulfate and mercaptoethanol respectively. As a result, proteins are single stranded polypeptides void of higher level structure and biological activity. Each simulated sample is placed in an electrophoresis gel sample well along with a well containing dyed-marker proteins and a positive control (mock gp-120 protein), all of known molecular weights.

Following electrophoretic separation of the denatured proteins from the three hypothetical culture extracts, Western Blotting is employed. In this relatively simple process utilizing paper towels, buffer solution, a polyvinylidene membrane, filter paper, and the gel block, the separated proteins are "lifted" from the gel block onto the membrane. The membrane is then stained for the presence of the proteins. It should be noted that immune identification of the transferred protein is not a part of this lab.

Proteins in the three patient samples are analyzed for their relative sizes based upon their position on the membrane (reflective of their original gel block position). In this exercise, two of the three patients should show a protein at the gp-120 position indicating a confirmation of

their HIV positive status, while one patient sample shows no protein band in the gp-120 position, and is HIV negative (Edvotek, 1998).

A graphical standard is established using semilog paper and drawing a best line generated by plotting known protein markers molecular masses on the y-axis versus their respective distances traveled (in electrophoresis) on the x-axis. Using this line, the mass of the suspect gp 120 can be determined.

As with earlier laboratory exercises, experimental data and its proper analysis, along with appropriate responses to lab analysis questions (Appendix D3), served as criteria for student evaluation.

Anaphylaxis and Transplantation Concerns

A teacher-generated series of labeled sketches were placed on the chalkboard as the chronology of sensitization and anaphylactic response were discussed. The immunology of localized and systemic responses was contrasted. The topic of desensitization and the experience of a personal allergy to codeine concluded topic coverage. Students were then asked to free write on this query, "Why is Buzz Allergic to Bee Stings?"

A final topic of discussion focused on immune reactions to transplantation. Various forms of grafts including: autografts, allografts, isografts and xenografts were explored. Varying antigenic strengths of tissues were pointed out with specific reference to the extremes of skin and corneas. The critical role of ABO and MHC matching was explored. Students were asked to explain the role of MHC

incompatibility in tissue rejection. This led to a discussion elucidating the actions of cytoxic T cells in tissue rejection and the genetic determination of major histocompatibility antigen types in siblings.

Our final topic in transplantation returned us to our genetic past while simultaneously opening the door to the future. Students were introduced to the concept of using transgenic pig organs for implantation in human beings. Prior to final testing, students were given a "be able to do" guide of objectives for the entire immunological unit (Appendix B7).

EVALUATION

Student generated laboratory results served as a major criterion for assessing the effectiveness of the immune instructional unit. An overview of individual laboratory results follows.

Ouchterlony Laboratory Results (Edvotek, 1998)

The following sketches depict idealized Ouchterlony laboratory results (figure 1). Plate one shows a reaction of identity with the antibody in the center well reacting to the same antigen in the outer four wells. Plate two shows a reaction of partial identity indicating that the antigens present in the the outer wells share some common epitopes that interact with the antibodies in the center well. The antigen in the upper right and lower left wells has a greater number of complimentary epitopes than the contrasting antigen in the upper left and lower right wells. Plate three shows a reaction of non-identity in which two different antibodies in the center well are interacting with their respective antigens independent of one another.

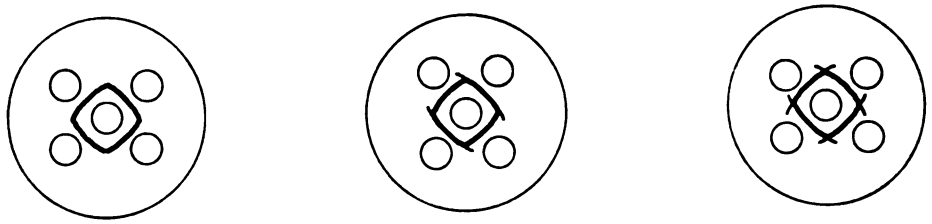

Plate One Plate Two Plate Three

Figure 1. Ouchterlony Plate Results

Twelve out of the twenty-one laboratory groups achieved ideal outcomes with eight of the groups showing partial success while the remaining two groups had poor results. Well loading technique was essential with a few groups overfilling wells which proved detrimental. It was noted that some students made poor well cuts with uneven edges and cracks in the agar preventing ideal final results and accurate interpretation. Laboratory analysis questions are in Appendix D3. Student question response performance is charted in Figure 2.

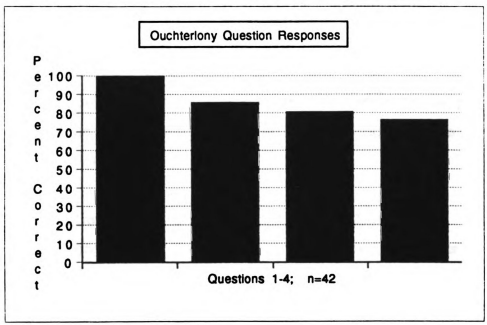

Figure 2 - Favorable Ouchterlony Question Responses

Student responses indicated a sound comprehension of molecular interactions and procedural structure. Further evidence of strong student performance is indicated in the lab grade distribution shown in Figure 3.

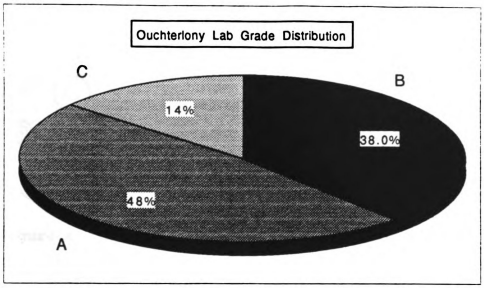

Figure 3 - Student Ouchterlony Lab Grades

Improvements in procedure include better instruction in well cutting and emphasis on proper pipeting. Some students were uncertain about the meaning of equivalence points and the relationship between precipitin band appearance and antibody-antigen concentration.

Radial Immunodiffusion Assay Lab Results

Eighteen of the twenty-one lab partnerships produced readable plates. The measurements taken from those plates yielded standard curves producing a variety of concentration estimations. It should be mentioned that student lab groups producing unreadable plates were given a readable set of plates from a peer lab group. Approximately fifty percent of lab groups were within +/- 10 percent of the true concentration of the antigen. Other responses were widely variable. An ideal plate result is pictured in Figure 4. Note the outside precipitin rings 1-5 are caused by known concentrations of antigens and the inside ring is the result

of an unknown concentration of antigen.

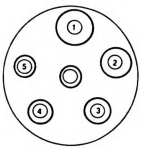

Figure 4 - Typical Student RID Plate Results (Edvotek 1998)

Responses to questions indicated a sound comprehension of laboratory concepts. A grade distribution of student performance which took into account original plate quality, graph quality,and lab analysis questions (Appendix D3) is shown in Figure 5.

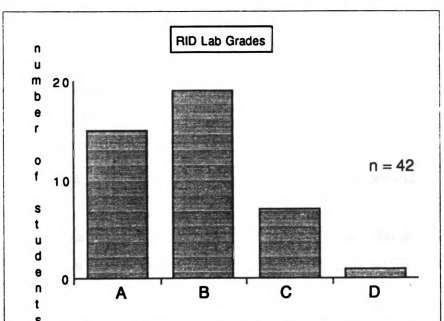

Figure 5. RID Lab Grade Distribution

Generally, because of the similarity between the Ouchterlony and RID lab set-ups, students performed notably

better in the areas of well cutting and pipeting. A major source of error in the lab came from improper measurements of precipitin rings diameters; due to a lack of concentration, and inferior procedure. Measurement accuracy will be improved in the future by using stereomicroscopes. This will directly improve measurements and elevate student performance. Student experiences and results were quite favorable.

Immunoelectrophoresis Lab Results

Ideal immunoelectrophoresis results are shown in Figure 6. Note that each precipitin arc designates a specific antibody-antigen interaction.

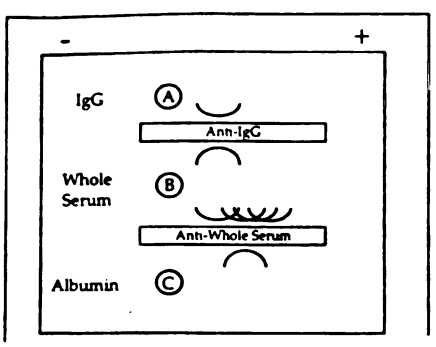

Figure 6. Immunoelectrophoresis Ideal Result(Edvotek,1998)

Due to a limitation of materials, nine lab teams of 4-5 students each were formed. Four of the nine stations produced excellent results. Three of the nine stations produced some degree of precipitin banding while two groups yielded no observable precipitin banding.

Student analysis responses reflected a sound comprehension of the molecular separations and antigen-

antibody interactions occurring in the lab. A few students missed the point of the tracking dye. The arc appearance of precipitin bands also confounded several students, probably because of their focus on the rectangular antibody wells.

Procedurally speaking, there were many more chances for error in this procedure compared to our earlier two investigations. The improper use of wicks to move the buffer across the surface of the gel may have caused some problems. A few groups made errors in either the formulation or pouring of their agar. At least one group added antibody to the trough prior to electrophoresis of antigens.

Again, I was generally pleased with the industrious and cooperative attitudes displayed by lab group members. For the most part groups showed a sense of purpose with a genuine desire to achieve good results. In all of these laboratory exercises, students have shown pride in their work and an outward display of satisfaction upon achieving sound lab results. Poor results spurred notable consternation. Figure 7 shows student performance in laboratory analysis questions (Appendix D3).

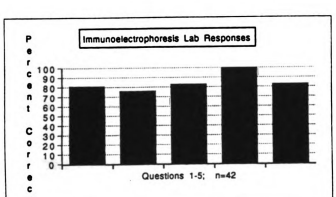

Figure 7. Immunoelectrophoresis Lab Question Responses

ELISA Systemic Lupus Diagnosis Computer Simulation Lab (HHMI)

Unlike engaging in wet labs, students who made procedural errors were able to start the investigation anew. Therefore, all students were expected to achieve ideal results in diagnosing the three hypothetical patient sera. The lab was programmed to yield a positive, negative, and inconclusive result.

Student assessment was based upon interpretation of final computer generated spot plates and responses to four laboratory questions (Appendix D3). The distribution of lab grades for this activity is shown in Figure 8. One student did not complete the lab report.

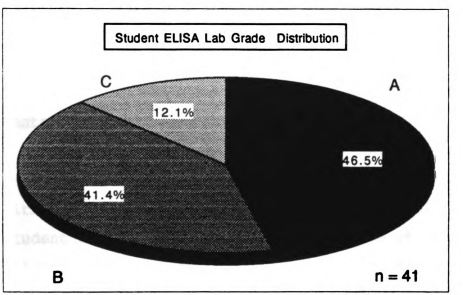

Fig. 8. ELISA Lab Grade Distribution

This being our third computer simulation of the year, students were somewhat familiar with the process. The reality of the SLE lab was excellent. Student comments and focus were very favorable. Students expressed an

appreciation for the program's level of instruction and background infor-mation. A glossary and a molecular pictorial representation of ELISA were most helpful and frequently referenced. Students showed confidence in their ability to understand the purpose of each manipulation in the lengthy procedure.

As a teacher, I appreciated the lack of a canned "correct" response every time the student clicked on the appropriate prompt. Students had to think in order to properly execute the lab procedure and gain valid results. An example of this was in pipeting. If the student was hasty in his/her measurements of various aliquots, the experiment was doomed. In the end, the ELISA-SLE simulation prompted students to assume the demeanor of a laboratory technician carrying out a critical procedure. I'm thoroughly pleased with the educational outcomes of this program!

Western Blot HIV Diagnosis Lab

After reading the description of this lab in an Edvotek sales catalog, I felt this would be an ambitious undertaking. With so many challenging steps in the protocol, the chance of student error was high. However, because of the lab's strong relevance to our immunology unit, I felt it was worth the effort. I also wanted students to perform a blotting procedure, something we had not done before.

Materials in this laboratory were limited, forcing students to work in five large groups of approximately eight students apiece. This group size was not conducive to ideal results. The "too many cooks in the kitchen", adage surely applied to our situation.

Only one group of the five obtained results suitable for analysis. In the failing groups, it appeared as though no protein made it through to the polyvinylidine membrane. I am suspicious of some groups' buffer formulations. One group also punctured their wells with micropipet tips causing a loss of sample.

All students utilized the same data and prepared the same graph. Our class molecular weight estimation of the standard protein and the glycoproteins in patient samples 1 and 3 was approximately 114,000 u. A distribution of student responses to the Western Blot analysis questions (Appendix D3) is shown in Figure 9.

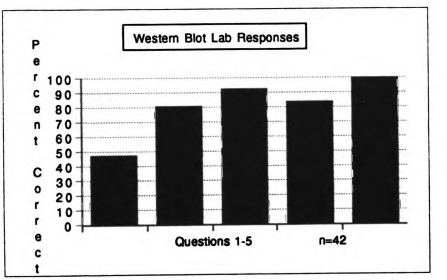

Figure 9. Western Blot Student Responses.

HCG-URINALYSIS TEST (Wampole, 1985)

Students were not responsible for laboratory reporting in this procedure (Appendix D4). However, the value of this exercise should be duly noted. When you mention to students that you are about to perform a scientifically reliable

pregnancy test, they are naturally very curious. The relevance and science embodied in the procedure provided an enjoyable and meaningful learning activity. After discussing the protocol for monoclonal antibody production, students were immediately able to apply the technology.

Pre and Post Test Statistical Analysis

The pretest and post test items (Appendices E1-E2) were identical, with the exception of three pretest short answer items being worked into the post test as a subset of more demanding questions.

Pretest performance indicated a relatively low entry level of knowledge. Of 62 possible points the pretest mean raw score was 15.2 which equated to a 24.6 % mean. The highest raw pretest score was 28 and the lowest pretest raw score was 7 out of 62. Individual subject pretest and post test performances are broken down in Table 2.

Comparable post test numbers showed a significant knowledge gain. The mean raw post test score was 47.2 of a possible 62 while the mean percent was 76.2, representing an increase of 51.6 percentage points over the pretest mean. The highest post test raw score was 58 and the lowest raw score was a disappointing 21 of 62 possible.

Utilizing a one-tailed test at a 0.05 level of significance, a z score was calculated. The sample size called for a z rather than a t test. The critical value for z in this test was 1.645. The calculated value of 33.7 statistically allows the sound rejection of the null hypothesis. The change between pre and post test scores is statistically significant and offers a degree of validation

Table - 2 - Breakdown of Individual Pre and Post Test

Student	Pretest %	Post Test %	Difference
1	34	92	+58
2	37	87	+50
3	18	68	+50
4	18	74	+56
5	24	63	+39
6	19	65	+46
7	15	40	+25
8	34	87	+53
9	29	79	+50
10	24	81	+57
11	34	88	+54
12	45	94	+49
13	29	82	+53
14	42	92	+50
15	32	73	+41
16	11	58	+47
17	13	74	+61
18	29	87	+58
19	16	34	+18
20	16	81	+65
21	19	77	+58
22	15	56	+41
23	23	79	+56
24	23	65	+42
25	21	77	+56
26	18	84	+66
27	19	76	+57
28	23	81	+58
29	26	81	+55
30	18	84	+66
31	23	66	+43
32	19	66	+47
33	24	81	+57
34	34	87	+53
35	26	87	+61
36	23	73	+50
37	26	68	+42
38	34	81	+47
39	26	84	+58
40	32	92	+60
41	29	92	+63
42	15	66	+51

for the immunology unit's instructional effectiveness. See Table 3 for a complete statistical analysis.

Pre and Post Test Item Analysis

An item analysis of the identical multiple choice selections from the pretest and post test is shown in Table 4. Some very important observations regarding the instructional unit's strengths and weaknesses were apparent.

Improved instruction in lymphoid tissue anatomy is an area of concern as indicated by the lack of improvement from pre- to post test on questions 3 and 9. This information was covered the first day of the unit. Lack of study and review on the part of students may be partially responsible for this lackluster change.

Question 14 regarding tumor specific antigens is very difficult because of the inclusion of both natural killer cells and cytoxic T cells. Rewording is required for clarity.

Questions 11 and 12 showed some degree of improvement, but a sizable number of students failed to recall the various forms of immunity and interferon's general function. Both of these items were covered in the introduction to nonspecific immunity lecture occurring during the second day of the unit. The role of interferon was not reiterated. This error of omission can be corrected.

The last test item of concern was question 17, a higher level thinking question, asking students to select an analogy most aptly describing the events of B lymphocyte activation. There are two choices that demand attention and students have trouble selecting the proper one. This is an effective

Table - 3 - Statistical Analysis of Pre and Post Test Scores

Student	Pretest %	Post Test%	Difference	Diff - Avg. Diff	(Diff. - Avg. Diff)^2
1	34	92	+58	6.4	40.96
2	37	87	+50	-1.6	2.56
3	18	68	+50	-1.6	2.56
4	18	74	+56	4.4	19.36
5	24	63	+39	-12.6	158.76
6	19	65	+46	-5.6	31.36
7	15	40	+25	-26.6	707.56
8	34	87	+53	1.4	1.96
9	29	79	+50	-1.6	2.56
10	24	81	+57	5.4	29.16
11	34	88	+54	2.4	5.76
12	45	94	+49	-2.6	6.76
13	29	82	+53	1.4	1.96
14	42	92	+50	-1.6	2.56
15	32	73	+41	-10.6	112.36
16	11	58	+47	-4.6	21.16
17	13	74	+61	9.4	88.36
18	29	87	+58	6.4	40.96
19	16	34	+18	-33.6	1128.96
20	16	81	+65	13.4	179.56
21	19	77	+58	6.4	40.96
22	15	56	+41	-10.6	112.36
23	23	79	+56	4.4	19.36
24	23	65	+42	-9.6	92.16
25	21	77	+56	4.4	19.36
26	18	84	+66	14.4	207.36
27	19	76	+57	5.4	29.16
28	23	81	+58	6.4	40.96
29	26	81	+55	3.4	11.56
30	18	84	+66	14.4	207.36
31	23	66	+43	-8.6	73.96
32	19	66	+47	-4.6	21.16
33	24	81	+57	5.4	29.16
34	34	87	+53	1.4	1.96
35	26	87	+61	9.4	88.36
36	23	73	+50	-1.6	2.56
37	26	68	+42	-9.6	92.16
38	34	81	+47	-4.6	21.16
39	26	84	+58	6.4	40.96
40	32	92	+60	8.4	70.56
41	29	92	+63	11.4	129.96
42	15	66	+51	-0.6	0.36
			2167		3938.12

2167/42 = 51.6 (avg. diff.);

Sum of (Diff.-Avg. Diff)^2/41 = variance; 96.05
Sq. root of variance = Standard Deviation; 9.80
z= Avg. Diff./ Std. Deviation over sq. root of 42; 36.5

Table- 4 - Multiple Choice Pre and Post Test Item Analysis

Question	Pretest # Wrong	Post Test # Wrong	# Change
1.	25	1	+24
2.	28	8	+20
3.	34	27	+7
4.	38	13	+25
5.	9	0	+9
6.	35	5	+30
7.	18	8	+10
8.	16	7	+9
9.	26	22	+4
10.	2	0	+2
11.	24	14	+10
12.	30	19	+11
13.	30	13	+17
14.	28	15	+13
15.	24	7	+17
16.	37	9	+28
17.	22	17	+5
18.	30	12	+18
19.	28	11	+17
20.	22.	9	+13

diagnostic question indicating a need for more detailed instruction.

Item analysis also included scrutiny of comparable fill-in, labeling, and short answer responses. Anatomical labeling improved from an average of 2.5 out of 8 items on the pretest to an average of 7.68 of 8 on the post test. The short answer questions regarding primary functions showed a tremendous improvement, from 3.1 out of eight items on the pre test, to 7.4 on the post test. Throughout the test, students excelled in areas concerning immune physiology.

The final area of comparative pre- and post test analysis came in the three short answer responses. Rubrics were established for each question, seven key rubrics for the first question, five for the second question, and four for the final question (Appendix E4). Each rubric was given a value of one point resulting in a total of fifteen points for the three short answer questions.

Proper responses to these questions required sound understanding of the immune system. As expected, students showed a tremendous improvement in post test versus pretest performances. An average score of 1 point out of 15 on the pretest leaped to a 14.3 point average on the post test. Short answer, process-oriented questions (often functional in nature) brought out the best in student performance.
The post test also served a second purpose: to determine individual student grades for the unit. Recall that the post test was expanded to provide a realistic assessment of unit content. A grade distribution is shown in Figure 10.

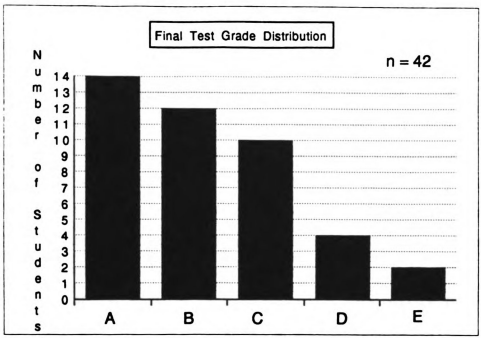

Figure 10. Final Immunology Unit Test Grades

CONCLUSION

The efficacy of the immune instructional unit has been statistically supported through analysis of student performance outcomes. This is expected and good; but as a teacher, I am much more impressed by the increased level of student enthusiasm and academic vigor sparked by the new instructional activities. With rare exception, students approached the learning activities and laboratory exercises with curiosity and zeal.

I am particularly pleased with laboratory performance. Ideal outcomes were frequently obtained. These outcomes are the result of numerous effective laboratory behaviors. Students regularly displayed higher level thinking skills and concept mastery in writing quality responses to lab analysis questions. Students added new laboratory skills and experiences to their repertoire. The lab activities provided valuable college preparatory lessons.

Team cooperation and cohesion were other qualities essential to laboratory success. Students performed very well in these areas generating excellent classroom morale. Greater student involvement, observed in the tremendous reduction of late and incomplete assignments, and the consistent and frequent application of higher level thinking skills were notable outcomes of the immunolgical unit. Ultimately, the immunology learning activities encouraged student behaviors and outcomes that greatly reduced the negative influences of senioritis.

Immunological learning activities encouraged the review and application of many course concepts. Students were

challenged to integrate old concepts with new insights. New learning activities encouraged students to forge sophisticated, functional understandings of biology. Many students generated detailed essay test responses indicative of higher level thinking and academic growth.

Student-initiated class discussions were frequent and lively. Shared experiences fueled discussions stimulating the review and application of general immune concepts. This is an offshoot of the relevance of immunology-related topics to high school seniors. On a related note, I am pleased that graduating seniors understand the science of HIV-AIDS.

Another key indicator of student acceptance was the decline in late and incomplete assignments in comparison to the previous three years. Only one student failed to hand a lab assignment. Remarkable!

Several areas of the unit need to be reassessed and revamped. The item analysis of the post test was particularly revealing. Improved instructional efforts in gross immune anatomy will be implemented. More specific objectives, improved organization, and added time on task will help in this area. It was duly noted that students performed weakest in test items over materials covered earliest in the unit. More frequent short quizzes may encourage better study habits and comprehension. A recommended brief nightly review would also be helpful. I may have overemphasized the "no homework" theme.

Another area for improvement is in time allocation. Students were encouraged to move through many activities at a brisk pace. For some, this was fine, but others need a little more time for assimilation. The unit content and

structure all but eliminated inattentiveness; however, a happy medium must be reached.

Incorporation of the laboratory exercise, Induction of Immunoglobulin Formation in *Periplaneta* (Appendix C), is slated for next year. This exercise and it's extension activity, "Vaccine Formation in *Periplaneta*", is also being considered for independent research projects (Reich, Karp, 1982). These activities will allow students practical experience with live animal research, as well as, further practical application of many concepts in basic immunology. Students who successfully induce antibody formation and later prepare a vaccine, will surely feel a sense of scientific achievement. This experience may impact students in later educational and career choices.

The new immune instructional unit raised the "academic" bar at a time when students were weary and sometimes uncooperative. However, with a sensitivity for homework limitation and the presentation of a thought provoking, variety of relevant learning activities, students ended the year on a productive academic note. The immune instructional unit measurably enhanced the educational experience of Dow High School's Advanced Biology students.

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APPENDICES

Appendix A1

Date _____

Dear Mr./Ms. _____

As a component of Michigan State University's Masters' Program in Secondary Science Education, I am required to construct and implement an instructional unit in a select area of modern biology. Furthermore, statistical analysis of student inputs and assessments will allow proper evaluation of the unit's effectiveness.

As a result of a Summer research course, I have constructed a series of integrated laboratory and classroom exercises in **immunology**. This unit will involve approximately two to three weeks of instructional time and is a basic course requirement. I sincerely feel the newly developed learning exercises will spark excitement and maximize student interest, participation, and proficiency in this highly active and relevant area of biology.

The instructional unit components involve the use of a variety of media including texts, computer software, periodicals and recently published Howard Hughes Medical Institute materials, as well as, teacher generated media. Laboratory components include;
*electrophoresis of bovine gamma globulins
*agglutination reactions of ABO antigens
*agglutination reaction of Human Chorionic Gonadotropin.
*precipitin reactions demonstrating antigen-antibody interactions
*induction of antibody formation in cockroaches
*creation of a vaccine in cockroaches
*investigation of soybean resistance to common soil mold

I am requesting permission to use your son or daughter's quiz, test, and laboratory results. Student inputs, individual responses and scores, will remain confidential and be expressed anonymously in all data analyses. Student confidentiality will be protected to the maximum extent of the law.

One laboratory exercise involves the use of micro quantities of bee venom. Any student with an allergy to bee stings will not be allowed to participate in this exercise. An alternative learning experience will be provided. Please note this on the permission form.

As with all of our laboratory investigations, safety is our number one concern. Students will handle any biologically active materials with gloves and the investigation involving saliva will mandate that students handle only their own saliva. An autoclave will be used in the disposal of any biologically active material. We exercise sound laboratory practices and safety measures.

If you have any questions or concerns regarding this instructional unit, or your son or daughter's participation, please contact me at 839-2482 or 835-8997. Thank you for your time and cooperation.

Sincerely,

Lee J. Koski
Advanced Biology Instructor
H. H. Dow High School

Mr. Koski, you have permission to use assessment data (quiz scores, test scores, laboratory reports) generated by _____(student). I understand that no student names will be used in the final analysis of this teaching unit. I also understand that student confidentiality will be protected to the maximum extent allowable by law.

_____(Parent or Guardian)

_____(Date)

*****Student allergy to bee sting: YES NO (circle one)

Appendix A2-Unit Outline and Objectives

Activity: Introduction to Immunology Learning Packet Objectives: -Awareness of Pathogens, -Recognition of Key Immune Breakthroughs and Terminology, -Knowledge of Jenner's Smallpox Vaccine, -Knowledge of Immune System Organ Anatomy Related Physiology (lymph system and lymph circulation, spleen, lymph nodes, lymphoid tissues, thymus, bone marrow)
Activity: Nonspecific Immunity-A Teacher Directed Lecture-Discussion Objectives: -Knowledge of the Functions of skin, mucosal barriers, acids, normal flora, nonspecific phagocytes, natural killer cells, interferons, complement system, lysozyme.
Activity: Ouchterlony Laboratory Investigation Objectives: -Execute the Proper Lab Procedures Required for Valid Results - Proper Interpretation of Precipitin Bands (or lack of)
Activity: Cells of the Immune System Learning Packet Objectives: -Recognition of B and T Lymphocytes, Macrophages, and the various other leukocytes, -Visualize the Chronology of Leukopoiesis, -Associate Corresponding Primary Functions to each Cell Type.
Activity: Examining Cells of the Immune System Laboratory Objectives: -Visual Identification of Various Leukocytes, (lymphocytes, monocytes, granulocytes) , Recognition of Pathology of Acute and Chronic Lymphocytic Leukemia
Activity: Key Molecules of the Immune Response Learning Packet Objectives: -Knowledge of the Source and Activity of MHC I, MHC II, CD4, CD8, Critical Receptors, Interleukins, Antibodies, Cytotoxins, -Recognition of Four Specific Actions of Antibodies. -Sketch and Label the Two-Dimensional Model of an IgG -Recognition of the Five Classes of Antibodies and their Associated Actions.
Activity: Flow Chart Centered Class Discussion; Clonal Selection Objective: -Sketch the General Process of Antigen Directed B-Cell Activation.
Activity: B-Cell Transduction Diagram Objectives: -Recognize the Role of Second Messengers, Kinases, Phospholipase, Calcium, and Transcription Factor in B-Cell Activation.

Activity: Monoclonal Antibody Reading and Flowchart Objectives: -Understand the Role of Non-Human (mouse) Cell, Multiple Myeloma Cell, Hybridoma - Outline the Sequence of Events Leading to Monoclonal Ig's - Specify Several Applications of Monoclonal Antibodies
Activity: Detection of HCG in Pregnancy Testing Laboratory Objectives: -Explain the Rationale Behind the Wampole Labs Positive and Negative Pregnancy Tests - Accurately Interpret a Positive Urinalysis for HCG
Activity: Scientific American Reading; "How the Immune System Develops" Objectives: - Cite (in writing) Five Newly Acquired Insights in Immunology
Activity: Small Group Discussion Following Campbell Text Reading Objectives: - Describe How a Relatively Small Amount of Genetic Material Can Code for a Large Number of Varied Immunoglobulins
Activity: HHMI Reading; "The Secret of Our Success" Objectives: - Summarize the Work and Conclusions of Tonegawa Regarding "jumping" Gene Segments
Activity: Radial Immunodiffusion Laboratory Objectives: - Execute the Correct Procedures Required For Valid RID Results - Determine the Unknown Concentration of an Antigen - Demonstrate a Knowledge of RID Applications
Activity: Class Discussion of Diagrams Depicting Specific Immune Responses to Three Different Pathogens Objectives: - Chronologize the Events of Macrophage, B Cell, and T Cell Responses to Influenza, Pneumococci, and Leishmania
Activity: Class Sketching and Discussion Depicting T-Cell Activation of B-Lymphocyte (Human Anatomy-Physiology Text by Marieb) Objectives: - Sketch and Label the Receptors Critical to Macrophage, T and B Interaction - Name and Assign Correct Functions to Interleukins
Activity: PreLab ELISA Antibody Construction Activity Objectives: - Using Scissors and Preformed Paper Components, Construct an Enzyme Linked Antibody. - Use The Paper Antibody to Show a Positive ELISA Test for a Hypothetical Paper Antigen

Appendix A2 (continued)

Activity: PreLab Internet Reading; "Systemic Lupus Erythematosus" Objectives: - Gain an Awareness of the Cause, Symptoms and Treatments for SLE
Activity: Laboratory HHMI Computer Simulated ELISA Based Detection of SLE Objectives: - Correctly Interpret and Carry Out Procedures for The Screening of Anti-DNA Antibodies Characterizing SLE - Correctly Interpret Spot Plate Results Indicating Positive, Negative, and Inconclusive Tests - Demonstrate an Understanding of the Molecular Interactions Featured in this Assay
Activity: Reading from HHMI; "The Killers That Save Us" Objectives: - Complete a One-Two Page Written Summation of the Article
Activity: Immunoelectrophoresis Laboratory Objectives: - Characterize the Protein Constituents in A Mixture Based Upon Their Antigen-Antibody Interactions - Execute Proper Procedures Necessary For Electrophoretic Separation and Natural Antigen-Antibody Interaction - Proper Interpretation of Precipitin Bands
Activity: Mechanisms of Autoimmunity-Reading Followed By Teacher Guided Discussion Objectives: - Cite Three Specific Scenarios Leading to Autoimmune Disease - Name and Describe the Cause and Symptoms of Three Autoimmune Disorders
Activity: Partner Activity: Use Campbell Text as a Resource for Learning the Events of the Life Cycle of HIV Objectives: - Make a Labeled Sketch of A Helper T Cell Being Successfully Infected by a viron of HIV - Identify the Role of Reverse Transcriptase - Learn the Chronology of Steps in the Life Cycle - Be Familiar with the Terms Budding, Provirus, CD4, gp 120
Activity: Laboratory Identification of gp 120 by Western Blotting Objectives: - Carry Out Western Blot Technique, Successfully ID gp120
Activity: Reading From Marieb Text-Immune Concerns in Transplantation Objectives: - Name Key Antigens in Histocompatitibility Tests - What is Cyclosporin Used For? - Describe the Meaning of GVH

Appendix B1

Introduction to Immunology Learning Packet

1798-Jenner	Cowpox lesion material to vaccinate against smallpox-beginning of modern vaccines
1879-Pasteur	Vaccines against chicken cholera, anthrax, and rabies
1884-Metchnikoff	Discovery of phagocytic cells
1890-von Behring, Kitasato	Discovery of antibodies in the blood-beginning of antitoxin therapy
1893-Ehrlich	Proves antibodies can work against toxic chemicals
1893-Widal	Uses concepts of antibody clumping to diagnose enteric diseases (typhoid fever)
1894-Rous	Uses horse generated antibodies in the form of antiserum to fight diphtheria
1904-Wright	Antibodies can coat cells leading to phagocytosis
1904-Koch	Develops tuberculin skin test
1912-Carrell	Work in organ grafting
1913-Richet	Studies in anaphylaxis (systemic allergic response)
1919-Bordet	Discovers immune proteins known as complement system

1930-Landsteiner	ABO blood groups, later Rh (allows successful transfusions)
1942-Coons	Fluorescent antibody staining
1947-Ouchterlony	Gel diffusion test for antibody
1953-Grobar	Electrophoresis for antibodies
1959-Edelman	Antibody molecular structure
1960-Burnet-Medawar	Lymphocyte activation-inactivation
1966-Ishizaka	IgE structure
1970-Yalow	Radioimmunoassay for hormone detection
1980-Snell	Genes for major histocompatibility complex (basis for tissue rejection)
1982-Jerne, Milstein, Kohler	Immune theories, monoclonal antibodies
1987-Tonegawa	Immunogenetics of antibodies
1990-Murray	Organ transplantation
1996-Doherty, Zinkernagel	T-lymphocyte specificity

Welcome to the World of IMMUNOLOGY!

Are you feeling good today? How's your health? If you're in tip-top shape, you've got your immune system to thank. If you are ill, odds are you'll feel better in a day or two. Again, say thanks to your immune system. This conglomeration of organs, cells and molecules is constantly on the look out for those things that could make you sick or disrupt your homeostatic state. Often the primary targets of the immune response are pathogens like viruses, bacteria, fungi, and protozoans. In a few moments we'll look at some notorious pathogens and parasites. Other times the immune response is directed against disease that seemingly springs from within; cancer.

Mankind's application of immune knowledge is relatively recent in our history. With previous knowledge of similar activities, Dr. Edward Jenner inoculated a little boy with the "juice" from cowpox lesions found on the hands of milkmaids. The little boy was later intentionally exposed to smallpox but did not come down with a serious case of the disease. Although Jenner had no idea of the internal physiology involved in the immune response, he began the first modern vaccination program. In fact, the word vaccination originates with Jenner stemming from the Latin word "vacca" meaning cow. So why was the little boy protected from smallpox, an often fatal disease? Stay tuned?

Advances in the 19th and 20th century have been numerous leading us to our current understanding of the immune system and its functions. With these immune understandings have come great advances in the prevention, diagnosis, and treatment of human and higher animal ailments. Vaccinations, allergy treatments, organ transplantations, and even blood transfusions are all the result of immune research and their resultant technologies.

STOP!

Let's familiarize ourselves with some common, nasty, sometimes even deadly pathogens and parasites; common targets of the immune response. Turn to the next page and carefully study the form (morphology) of these disease causers. After you've examined a given pathogen, try to classify it as virus, bacterium, protozoan or worm. Check your guesses with the numerical key following the scanning electron micrographs.

Pathogen Key

1. *Trichomonas*-a protozoan
2. *Ascaris*-multicellular intestinal roundworm, male and female
3. *Treponema pallidum*-spirochete bacterium causing the venereal disease syphilis
4. *Adenovirus*-virus of the common cold
5. Influenza virus-
6. *Streptococcus pneumoniae*-bacterium causing lobar pneumonia
7. *Staphylococcus*-bacterial cause of various staph infections
8. Rubella virus-cause of the German Measles
9. *Mycobacterium tuberculosis*-bacterial cause of tuberculosis
10. Rabies virus-viral cause of rabies
11. *Proteus mirabilis*-bacterial infections in wounds and urinary tract
12. *Helicobacter pylori*-bacterium causing peptic ulcers
13. *Actinomyces*-bacterium causing dental disease
14. *Neisseria gonorrhoeae*-bacterial cause of gonorrhea
15. Polio virus
16. Scolex, the head of a tapeworm-this mulitcellular parasitic intestinal worm shows four suckers and many hooks for attachment
17. Rotavirus-cause of acute intestinal infections in young children
18. Ebola virus-recent cause of deadly epidemic in Africa
19. *Clostridium tetani*-bacterial cause of tetanus
20. *Herpes simplex* virus-cause of herpes infections
21. *Escherichia coli*-generally normal flora bacterium of intestinal tract, however; some strains are very pathogenic
22. *Legionella*-bacterium that causes Legionnaire's Disease
23. *Vibrio cholerae*-bacterial cause of cholera
24. *Listeria*-bacterium causing meningitis
25. *Hepatitis B* virus-
26. *Salmonella*-bacterial cause of food poisoning
27. *Bordetella pertussis*-bacterial cause of whooping cough
28. Cytomegalovirus-virus that can harm fetus
29. *Schistosoma*-a multicellular parasite that causes schistosomiasis

The Human Immune System

Which body organs and parts are components of the immune system? Go ahead and take a stab at this question----responses below.

Now take a look at the outlined diagram below.

Can you name the numbered body parts? Try it.

1.	2.
3.	4.
5.	6.
7.	8.

In the space below, assign a general function to each of the organs listed above.

Now turn to the next page for a review of key functions.

See next page.

1. Adenoids 2. Tonsils 6. Peyer's Patches-these are secondary lymphatic tissues referred to as ***gut associated lymphoid tissues.*** These tissues are strategically located within the body encouraging the trapping of antigens (foreign molecules) and cells that bear antigens. These tissues are loaded with T and B lymphocytes.
3. Lymph Nodes-These are masses of cells that filter lymphatic fluid (fluid originating from blood capillaries and moving into the spaces between cells is collected by lymph capillaries and vessels that direct the fluid to lymph nodes) and serve as a germination center for B and T lymphocytes. A gram of lymph node tissue may contain as many as a billion lymphocytes. Antigen presenting cells and lymphocyte activation are common in lymph nodes. Lymphocytes and their products flow from the lymph nodes through lymph vessels and ducts to reach and circulate in the bloodstream. Note the position of lymph nodes. Although they are scattered throughout the body, they are prominent in the head-neck, armpit and groin regions.
4. Thymus-This organ is a primary lymphoid tissue. It is responsible for programming the making of T-lymphocytes. (What do you think the T of T-lymphocyte is all about?) The thymus is a critical producer of hormones important to immune development and response. They thymus plays a role in setting up and organizing the immune system of an individual. This organ is also important in programming self-recognition; the ability of the immune response to direct its fury at outsiders and not one's own tissues.
5. Spleen-This organ is a secondary lymphoid organ. It serves as a center of B and T cell germination and activation. The spleen is a blood filtering organ much like the lymph node is a lymph fluid filter. The spleen of an organism contains a cross-section of the general types of B and T lymphocytes characterizing the "beast."
7. Lymphatic Vessels-are critical to the flow of lymphatic fluid and lymphocytes and their products to and from the bloodstream.
8. Bone Marrow-like the thymus, bone marrow is a primary lymphoid tissue. It is the source of the parent cell of all immune cells. This "pluripotent"stem cell can give rise to any of the blood cells, including B and T lymphocytes and the antigen presenting macrophages. The true origin of the immune system lies in the bone marrow.

Activity Summary

Take a few moments and consider the information you have just processed. Respond or carry out each directive to the best of your ability. Try to do this without going back to the information presented, but if you feel stumped, go back and check out the information to gain a clear picture.

1. In a single sentence, describe the basic aim or function of your immune system.

2. What general lifeforms are responsible for causing disease? (Recall the SEM photos).

3. Of the diseases pathogens and parasites pictured and described, which was most intriguing to you? Why?

4. You are an experienced research scientist with unlimited laboratory resources. Which pathogen induced disease do you choose to conquer? Why did you select this specific disease?

5. The primary lymph organs are the _____ and _____. These organs are responsible for generating the cells that will seed all lymphoid tissues.

6. An experimental mouse is developed to born athymic (no thymus). What critical immune cell type are they unable to produce?

7. The gut associated lymphoid tissues include the _____, _____, and _____. These tissues serve as centers for antigen trapping and B and T cell germination and activation. Why might your lymph nodes swell in the presence of an infection? Think about this. No need for a written response here.

8. Based upon your limited introduction, why might a bone marrow transplant be a cure for certain forms of leukemia?

9. Quick! Name the organs of the immune system:_____
_____
_____

Appendix B2

Cells of the Immune System

All cells of the immune system have one thing in common, the same parent! Immune cells are derived from the **common stem cell**, sometimes called the hemocytoblast. This cell is born and replenished in the bone marrow. While still in the bone marrow, the parent cell begins to change or differentiate developing into any one of several intermediate cell forms. These intermediate cells then mature becoming one of the mature cells within the flow chart.

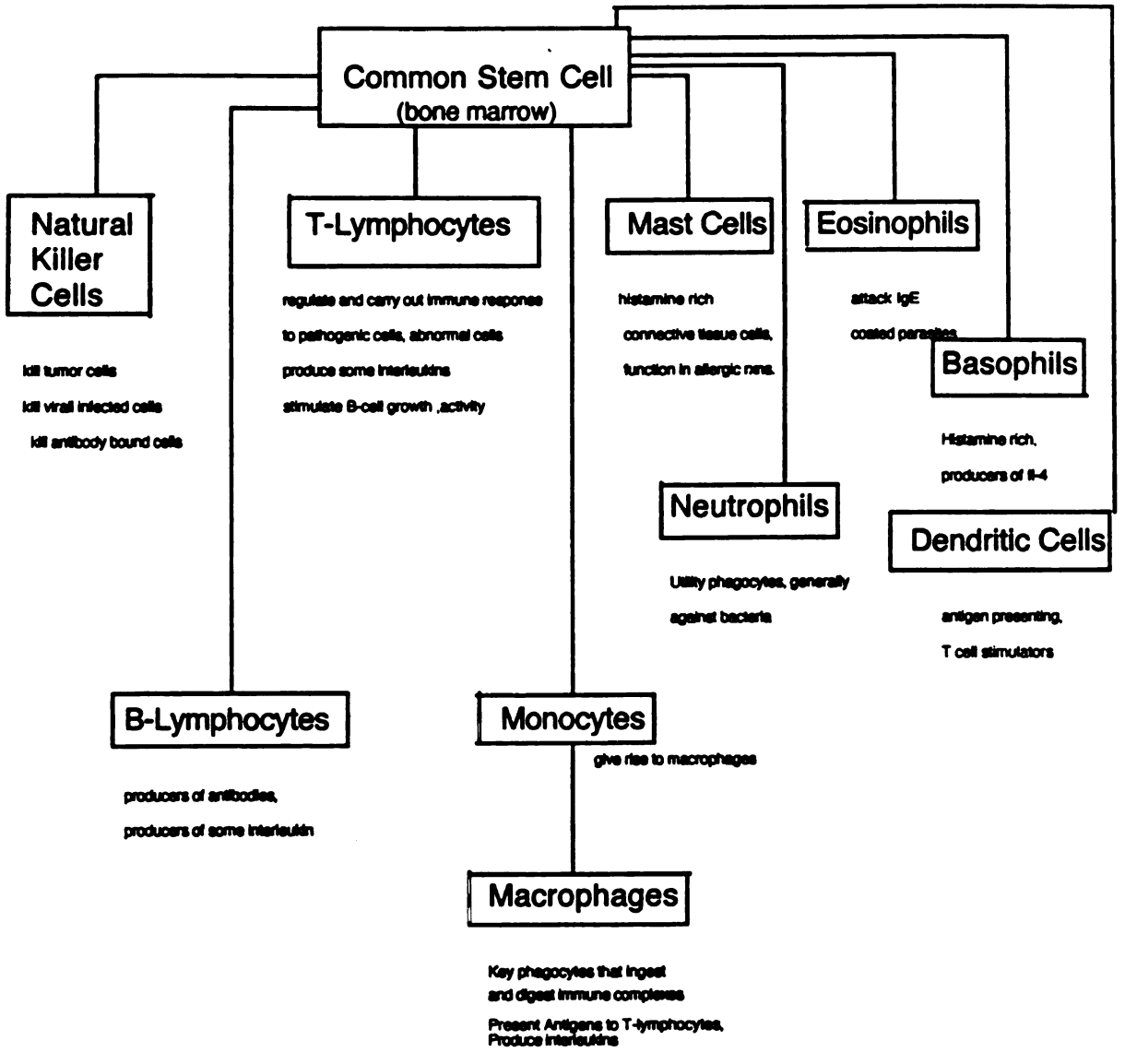
As an introduction to the cells of the immune system, examine the flow chart noting cell names and functions. Do this now please. Did you see cells that interacted with one of more other cells? Look for these functional relationships.

Wow! That's a bunch of cells and a whole lot to remember. Don't worry about remembering all of these cells and their functions now. We will gradually work our way up to this level of understanding. For now, let's just establish some basic concepts. Return to the immune cell flow chart and focus on **B** and **T lymphocytes.** Although all of the cells listed are important to normal immune function, the lymphocytes are the backbone of the immune response. T lymphocytes function in what is known as **cellular immunity.** This means that T-lymphocytes direct a cellular attack against foreign of abnormal cell types. These cells are recognized by the T-lymphocytes as nonself.

The B lymphocytes function in **humoral immunity** directed at specific foreign molecular components. The foreign molecules are generally referred to as antigens. Antigens may be part of a bacterium, virus, or other foreign cell or they may be free chemicals. B lymphocytes fight antigens with protein molecules known as **antibodies** or **immunoglobulins.** These specialty proteins are designed to chemically combine with and inactivate the very **antigens** that triggered their synthesis. If the antigen is a bacterial toxin, then the toxin is destroyed. You get the picture, antibodies inactivate foreign molecules called antigens. If the antigen happens to be a component of a pathogenic cell, then the antibody-antigen complexes will often lead to the death of the pathogen.

Return to the cells of the immune system flow chart and review names and functions.

Now let's get a closer look. See the diagrams of cells and the following flow chart. Match names and functions with the pictured cells. Make a quick but accurate pencil sketch of each cell next to it's name on the chart.


Common Stem Cell
(bone marrow)
Natural Killer Cells
kill tumor cells
kill viral infected cells
kill antibody bound cells
T-Lymphocytes
regulate and carry out immune response
to pathogenic cells, abnormal cells
produce some Interleukins
stimulate B-cell growth ,activity
Mast Cells
histamine rich
connective tissue cells,
function in allergic rxns.
Eosinophils
attack IgE
coated parasites
Basophils
Histamine rich,
producers of Il-4
Neutrophils
Utility phagocytes, generally
against bacteria
Dendritic Cells
antigen presenting,
T cell stimulators
B-Lymphocytes
producers of antibodies,
producers of some Interleukin
Monocytes
give rise to macrophages
Macrophages
Key phagocytes that ingest
and digest immune complexes
Present Antigens to T-lymphocytes,
Produce Interleukins

Appendix B3

Key Molecules of the Immune System

Now for the tough stuff! The molecules of immunity are many and varied. Historically, antibodies and complement were the first "immune molecules" to be understood. Since this time many other immune compounds have been discovered. In the past two decades, several Nobel Prizes have been awarded for work in this area. Many of the key molecules belong to macrophages and lymphocytes. Many of the molecules are involved in the uptake and response to antigenic substances. Some of the molecules coordinate T and B lymphocyte proliferation, differentiation and actions.Finally, specific immune molecules allow the destruction of invaders and/or their chemical toxins. Molecules characterizing immune function may be classified in the following categories:

Cell Surface Receptors and Signal Transducers-These molecules allow the detection of extracellular signals and the communication needed to spark a normal immune response. Some of the key surface receptors are known as CD molecules. CD4 and CD8 are critical CD receptors marking helper and cytoxic T cells respectively. Unfortunately, HIV recognizes and binds to CD4 receptor sites resulting in the uptake of virions by helper T lymphocytes. B lymphocytes also have their characteristic CD markers allowing them to interact with helper T lymphocytes. Another key group of cell surface marker molecules are the MHC's (major histocompatibility complex). In the immune response, MHC-I and MHC-II are critical to macrophage, T-cell, and B-cell interactions.

A variety of transducer molecules exist within the membrane and cytoplasm of T and B lymphocytes, macrophages and dendritic cells. Notorious are the G-proteins which behave as second messengers leading to the specific cellular response we associate with the extracellular molecule.

Interleukins-these are molecules produced by immune cells that allow the growth, proliferation and activation of immune competent cells like B and T lymphocytes. Many different interleukins have been discovered. These molecules allow various immune cells to communicate and coordinate their activities.

Antigen-Pathogen Inactivators-antibodies and cytotoxins, like perforin, are molecules that cause the inactivation of antigens or death of cells that possess antigens. The complement system of serum proteins is also a member of this group. This molecule "stuff" is heavy duty and really is the primary area of current immune research. Again, acquaint yourself with terms and ideas. Read content a few times, attempt to visualize cells and molecules "doing their thing". We will apply the critical cellular and molecular players to practical immune responses shortly.

Key Molecules of the Immune System (continued).

Please read the following with a desire to comprehend! This will give you an improved understanding of concepts on the previous page. Take your time, don't hurry, don't worry. Visualize!

1. Molecules like the CD receptors on B and T lymphocytes allow the uptake of chemical cues from outside the lymphocyte. These cues kick of a chain of events that move through a series of membrane molecules causing changes that are communicated to the cytoplasm and often the nucleus of the cell. These internal chemical changes lead to functional changes in the cell that we associate as being caused by the initial external chemical cue.
   Let's make this general scenario applicable to the immune response. A specific chemical antigen will "sit" on a specific B-lymphocyte receptor. This will lead to a chemical cascade of reactions that trigger B-lymphocytes into the active state. Rapid reproduction of B-cells ensues. These activated B-lymphocytes will then differentiate into plasma cells that begin to produce and secrete many copies of the specific antibody that targets the original stimulating antigen.
   There are many surface receptors and chemical transducers involved in even the simplest of B and T lymphocyte responses. We will explore these in due time.
2. Interleukins are the result of specific signals being transduced (communicated) to lymphocytes and macrophages. The word interleukin means "between leukocytes or white cells". These molecules move from one immune cell to another stimulating growth, differentiation, and the production and secretion of immune molecules (like antibodies and perforins). The interleukins allow coordination and cooperation between the cells of the immune system. There are many interleukins and more are likely to be discovered. Several interleukins are used in therapies because of their anti-cancer or anti-microbial properties. We will only look at a few interleukins in the context of their function during B and T cell activities. See the table on the following page.
3. Antibodies and cytotoxins such as perforins, are the inactivators of the agents of disease. Antibodies are produced by differentiated B cells known as plasma cells. Perforins are produced by killer T cells and ooze onto their cellular targets causing their ultimate death. We will look at these molecules shortly.

A QUICK REVIEW OF THE MOLECULES OF THE IMMUNE RESPONSE

1. Fill-in.

Molecules essential to the immune response might be grouped in one of three categories: the cell_____ markers and signal _____, interleukins, and _____ and cytotoxins.

2. Respond to the following:

CD molecules function as surface receptors...

3. Answer the following question as completely as possible.

Describe some of the sources and actions of immune molecules known as interleukins.

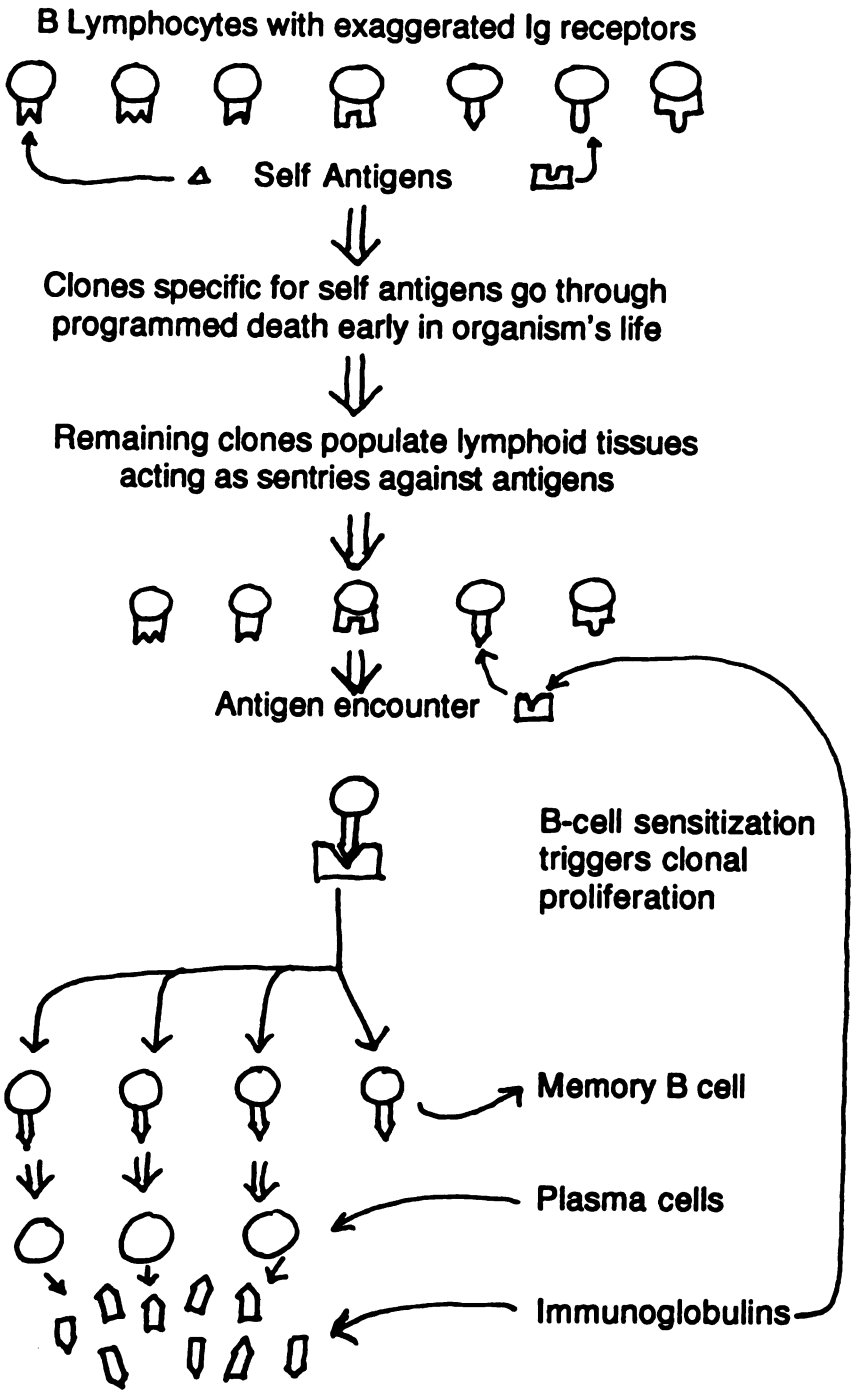
4. See the table of interleukins. Read through it to gain a general overview of interleukin actions. Do not try to memorize all the details instead, strive for a general overview.

5. Describe the source and actions of antibodies.

6. Describe the source and action of perforin.

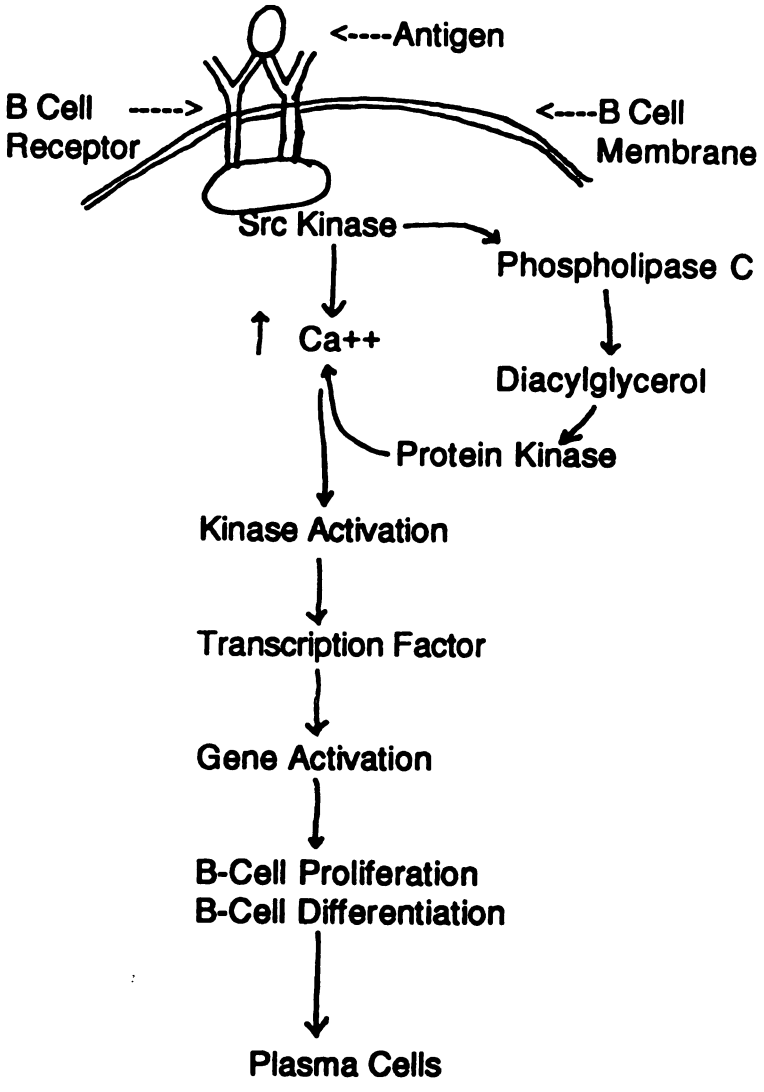
Appendix B4

Clonal Selection of B Lymphocytes



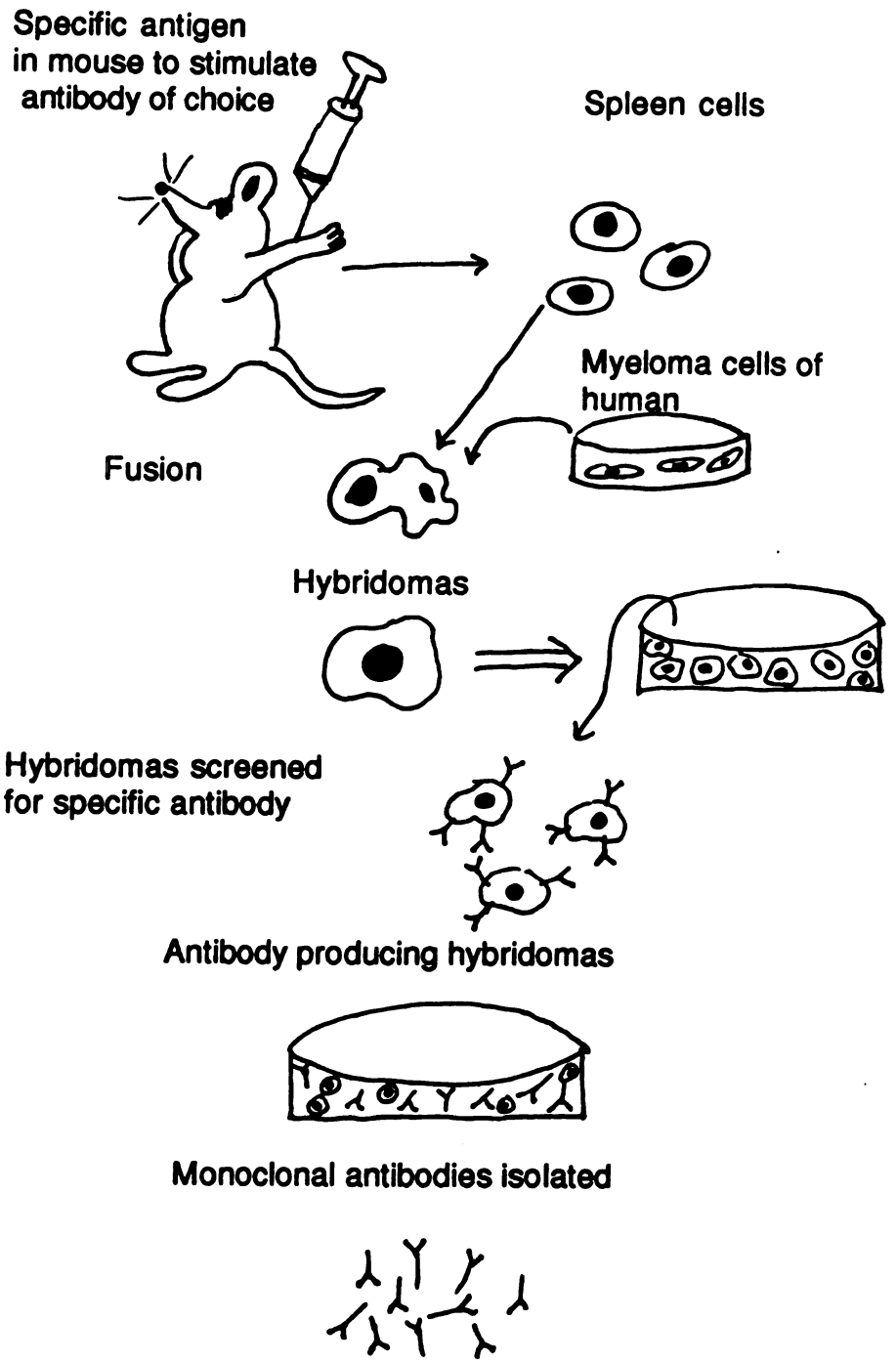
Appendix B5

Signal Transduction in B Lymphocytes



Appendix B6

Monoclonal Antibody Production
(Genentech Access Excellence 1989)



Appendix B7

Immune Test Objectives.

1. Be able to name the primary organs of the lymphatic system.
2. Be able to trace the general flow of lymphatic fluid from a point in the body to the circulatory system.
3. Be able to:
   a) give the general structure and function of the lymph nodes, the spleen, and lymph nodules.
   b) describe the major components of the physical and chemical nonspecific immune response-skin, gastric juice, mucous membrane, lysozyme, complement, interferon, normal flora, phagocytes, natural killer cells, etc.,
   c) describe the major components of the specific immune response-
      T-cells, B-cells, memory cells, helpers, killers, plasma cells, suppressor cells, APC's
   d) Describe the general role of MHC I, MHC II, CD-4, CD-8 in the immune response.
   e) Describe the general events of direct B-cell activation (recall clonal selection and B-cell transduction), and T-cell mediate B-cell activation.
   f) Describe the general genetic gameplan in coding for various clones of B and T cells.
   g) Be able to sketch and label the general structure of an antibody.
   h) Name the five classes of antibodies.
   i) Describe four different actions of antibodies.
   j) What is indicated by the term titer?
   k) Distinguish passive and active immunity. What is colostrum? What form of immunity is conferred by colostrum?
   l) Describe the general origin of B and T cells.
   m) What form of immunity is instilled by a vaccine?
   n) How are monoclonal antibodies produced? What are several uses of monoclonal antibodies?
   o) Describe the general event of an allergic reaction.
   p) What is anaphylaxis?

q) Describe the mechanics of ELISA-What are some uses for this process?
r) What are pyrogens? Where do pyrogens come from?
s) What are interleukins? Where do interleukins come from?
t) Describe the way in which some vaccines are produced.
u) What is RID, the Ouchterlony Test?
v) What is the second set or anamnestic response?
w) What is anaphylaxis? How does this situation arise?
x) State three different mechanisms responsible for the development of autoimmune disease.
y) Describe methods used in vaccine production.
z) Describe the general typing procedures used in tissue transplantation.

aa) Be certain that you have reviewed the earlier objectives presented in the unit overview.

Appendix C1

Laboratory Exercises With *Periplaneta*

Induction of Immunoglobulin Formation in *Periplaneta*

Discussion: Organismal immune reactions to foreign chemical agents (antigens), demonstrating immunological memory have long been considered a feature of vertebrate life forms. Recent work by Karp and Reins (1985-1990) has validated the existence of a humoral immune response in the resilient life-form *Periplaneta americana*, the American cockroach. Further efforts by William Yerkiewicz (1993) have made the demonstration of cockroach immune response feasible for classroom investigation. Prior to this investigation be sure that you are familiar with the general stimulation, form, and actions of vertebrate antibodies.

Objective: Stimulate the formation of a specific humoral response to bovine serum albumin (antigen). Verify the existence of antibodies against bovine serum albumin with immunodiffusion plate studies.

Materials:
- *Periplaneta americana*
- bovine serum albumin
- 1.0 mL syringes with #30 needles
- 0.75% saline
- Cockroach living quarters-sealble plastic containers with cedar chips, petri dish + sponge for water, dry dog food, vaseline barrier rimming plastic container wall. These animals are extremely quick and notorious breeders, do not allow escape-stay tuned for recommendations.
- immunodiffusion media-tris buffer + agar
- 35 mm Petri plates
- KCl
- small cork borer or straws

Procedure: Cockroach Handling and Care: The American cockroach is a large species typically reaching 3 or more centimeters in length and massing out at 1.5 to 2.0 grams at maturity. The animals are sprinters with short flight ability making them evasive and difficult to capture. Keep this in mind when housing and handling the animals. Smooth plastic containers with lids that snap tight work well. These may be lined with bedding materials like cedar chips. The upper inner wall of the container should be lined with petroleum jelly or a similar sticky substance. A petri dish containing a piece of new sponge and water along with dry dog food should take care of nutritional concerns.

Cockroaches are easily handled if they are chilled in the refrigerator for 20-30 minutes. Once slowed down, they may be captured for anesthesis. Five minutes in the freezer should immobilize them. Do not over freeze.

Albumin (antigen) and Control Injections:
0.10 gram of bovine serum albumin crystal is dissolved in 10.0 mL of 0.75% saline. 0.75% saline serves as the control injection.

Injecting Cockroaches to Induce Humoral Immunity:
Inject six mature roaches with 0.1 mL of 0.75% saline per gram of body mass. Injections should be made between the 4th and 5th abdominal sternites with a horizontal-anterior needle direction. Successful injection is noted by abdominal swelling and a lack of leakage.

Inject six mature roaches with 0.10 mL of bovine serum per gram of body mass. Use the techniques employed with the controls. Provide separate but identical housing for the control and experimental roaches. Injected roaches should be allowed to rest and revitalize (hopefully produce antibody) for ten days.

Demonstration of Humoral Immune Response:

-Dissolve 1.0 gram of noble agar in 100.0 mL of 0.6 M KCl and 0.05 M TRIS buffer (pH=7.5).
-Upon boiling, allow the agar to cool to 55 degrees Celsius and pour into 50 mm petri dishes to a depth of 5 mm.
-Allow the plates to solidify for at least thirty minutes (refrigeration hastens the time) and then use a sterilized cork bore or plastic straw to cut 7 circular wells 3 mm in diameter.
-Place the control and experimental cockroaches in the freezer for five minutes to anesthetize.
-Use a syringe to withdraw hemolymph from the abdomen. This fluid may need to be chilled to prevent coagulation. Withdraw enough fluid to fill the center well of the diffusion plate. This may require a few control and experimental animals. Do not overfill the wells. Bring it level to the agar's surface.
-Add hemolymph to the center of the control and experimental plates.
-Surrounding wells receive three different concentrations of bovine serum albumin, casein, myosin, and horse serum albumin.
-Suggested bovine serum albumin concentrations are 1 microgram per mL, 0.5 microgram per mL, and 0.1 microgram per mL. All other proteins can be made at 1.0 microgram per mL. Use 0.75% saline as the solvent.
-Cover the plates and place them in a moist, room temperature environment for twenty four hours. This can be done by placing the plates in a tray covered with moist paper towel and plastic wrap.
-Precipitin bands appearing as white bars are indicative of antibody formation. Observe all plates noting and sketching any visible results.
-Compare your results with those of several other lab groups. Record similarities and differences.

Questions for Analysis:

1. Identify the antibody and antigen in this experiment.
2. Use a series of labeled sketches to illustrate the concept of clonal selection and the induction of the specific antibody against serum albumin.
3. Sketch a typical IgG molecule and label the heavy chains, light chains, constant regions and variable regions.
4. Describe the common fate of antigens that are bound by antibodies.

5. Discuss notable similarities and differences in immunodiffusion plate results. Give plausible explanations and reasoning supporting the variable results. Focus upon experimental procedures and immune mechanisms.
6. Could it be possible that an experimental cockroach has produced the desired antibody, yet immunodiffusion results are negative? Discuss.
7. The cockroach is notorious in nature for being an evolutional survivor. Various cockroach species have been in existence for hundreds of thousands of years. How is the evolution of a humoral immune response an added survival benefit to the cockroach?

Vaccine Production In *Periplaneta*

Discussion: The development of vaccines represent landmark medical advances that have significantly influenced the average life expectancy of humans. At the turn of the 18th century, Dr. Edward Jenner utilized the less virulent cowpox virus as an immune stimulator creating resistance to its deadly "cousin", the smallpox virus. In fact, the word vaccine has its roots in Jenner's work as "vacca" refers to cow in Latin. Throughout the twentieth century, vaccines conquering many potentially debilitating and deadly pathogens have been developed and hailed as some of mankind's greatest scientific victories. Dr. Jonas Salk's development of a polio vaccine still stands as one of the most publicized and revered discoveries ever! Salk's success came when polio virions were treated in formalin. Current vaccine efforts are underway to conquer HIV and varying forms of cancer. Efforts by many investigators, particularly Dr. Stephen Rosenthal, have led to monumental breakthroughs in cancer therapy against previously incurable forms of melanoma.

There are many techniques for vaccine formulation. However, the general principle is always the same: find an agent that will stimulate a specific immune response to a desired antigenic carrier without inducing the disease. In the world of vaccine formation the concept of attenuation, the loss of pathogenicity while maintaining, is often the goal. This leads to the ultimate prize, a functional vaccine.

Objective: Produce and test a functional vaccine toward a toxin.

Materials:
-*Periplaneta*
-Honey Bee Venom (other venoms are available)
-0.75% saline solution
-1.0 mL syringes with #30 needles
-Suitable housing and feed for cockroaches

Procedure: (Refer to earlier discussions regarding the care and handling of *Periplaneta*).

- Prepare honey bee venom by dissolving the solid in 100 mL of a 0.75% saline solution yielding a 10 mg/mL concentration.
- Inject at least five cockroaches with 50 micrograms of toxin per gram of body mass. Return these cockroaches to their housing and observe over the next several days.
- Inject at least five cockroaches with 0.1 mL of 0.75% saline solution. Utilize these animals as controls.
- Place one-half of the remaining toxin solution in a loosely stoppered test tube. Place this test tube in a hot water bath at 80 degrees Celsius for 90 minutes. This heating should alter the venom molecules leading to their attenuation.
- Inject five cockroaches with 50 micrograms of cooled attenuated venom for each 1.0 gram of body mass. Allow these animals six to nine days to respond to the heat treated venom. Following this time period, inject each animal with 50 micrograms per gram of body mass of non-heated toxic venom. Compare all groups of animals noting noting general behaviors, life, death, and times of responses. You may wish to identify each animal individually with some form of external marking on the wings. Various markers will work.

Laboratory Report.

1. Prepare a brief abstract highlighting specific laboratory activities and results. Three to four sentences is ample length.
2. Include a listing of the materials necessary to successfully carry out this study. Be precise including housing and handling materials.
3. Provide a chronological listing of procedures.
4. Write an observation and data summary. Include data tables.
5. Summarize your experimental results and their ramifications in a "conclusions" section.
6. Answer the following questions:
    a) Using your own words and explanations, explain the basic mechanism of a vaccine.
    b) Define these terms: attenuation, toxoid
    c) Specifically, how is the vaccine against hepatitis B produced?
    d) Do vaccines stimulate cell mediated or humoral immunity?
    e) Do vaccines stimulate passive or active immunity?
    f) Why is it so difficult to formulate vaccines against certain pathogens such as the common cold virus and HIV?

Appendix D1

Laboratory Activity: Examining Cells of the Immune System

1. Obtain a microscope and human blood slide.
2. Clean the scope lenses and slide using lens paper.
3. Examine the slide under 100 X. Notice the vast majority of pink-red cells. These are the red blood cells or erythrocytes. Their primary function is to carry oxygen and allow for carbon dioxide transport and release.
4. Concentrate on the rather sparsely populated cells with the dark (purple) staining nuclei. These are white cells or leukocytes.
5. Refer to the diagram of stained blood cells.
6. Increase the power of magnification to 400X and begin to examine microscopic fields in the upper left of your blood smear. Move to new fields of view in a systematic manner so that the entire slide will be viewed without duplicating field observation.
7. When you encounter a leukocyte or white blood cell, try to identify it. This is something that can readily be accomplished after a little practice and using the blood cell handout.
8. Now count 50 different leukocytes. As you count these fifty cells, classify them as a lymphocyte, monocyte, neutrophil, eosinophil, or basophil.
9. Convert your counts to percentages of white blood cell types; i.e., if you found 8 monocytes out of the fifty total cells, then your monocyte percentage is 16.
10. Look to available resources to see normal distributions of white blood cell types. Does your slide show a normal distribution? Support this with numbers.
11. Can you discern a T lymphocyte from a B lymphocyte in this experiment?
12. Recall and describe the general role of the T cell in immunity.
13. Recall and describe the general role of the B cell in immunity.
14. When would you expect T and B cell numbers to rise?
15. Assign a basic function to:
    a) neutrophils-
    b) monocytes-
    c) eosinophils-
    d) basophils-
16. Examine a prepared slide of ALL (acute lymphocytic leukemia). Write a general statement comparing this blood slide to the "healthy" sample viewed earlier.
17. What might be some of the problems or symptoms of the individual owning ALL blood?

Appendix D2

Ouchterlony Lab-A Sample Procedure (Edvotek, 1989)

Agarose Preparation

1. In a 500 mL flask, add powdered buffer to 225 mL distilled water.

2. Add agarose packet to flask and swirl, avoid clumps.

3. Cover flask with Saran wrap and microwave in 20-30 second bursts until a full boil is reached. Do allow this to boil over.

4. Cool the agarose to 55 degrees C, the carefully pipet 5.0 mL of agarose into each of the three plates. Avoid bubbles, gently rotate plate to evenly spread agarose.

5. Allow solidification time and then mark the bottoms of the plates.

6. Use the template provided and set it under the plates. Use the hard plastic "straw" to cut wells in the pattern illustrated by the template. Remove agarose plugs.

7. Obtain samples A (animal antiserum), B (animal serum), C (albumin antigen), and D (Ig antigen) in their respective microtest tubes.

8. Micropipet 30 microliters of each sample in the following manner: **DO ALL TRANSFERS OF A SINGLE SINGLE REAGENT TYPE BEFORE DISCARDING MICROPIPET TIP. NEW SAMPLE, NEW TIP!**

   Plate One: 30 microliters of B in all four outer wells, center well 30 microliters of A.

   Plate Two: 30 microliters of B in two diagonally located outer wells, 30 micro liters of C in two remaining diagonally located outer wells. 30 microliters A in the middle well.

   Plate Three: 30 microliters of C in diagonally located outer wells, 30 microliters of D in remaining outer wells, 30 microliters of A in the middle well.

9. Be extremely careful not to jostle plates in any way causing a spillage of reagent.

10. Use a small plastic dish and moist paper towels to create an incubation chamber. Incubate labeled plates at 37 degrees Celsius for 48 hours.

11. Remove and observe for precipitin line or bands. Be diligent in your observation, proper lighting is essential. Record your results.

12. Prepare sketches of each plate along with an assessment of the type of reaction shown.

13. Respond to lab questions.

14. Discard plates upon instructor completing observations.

Appendix D3

Student Questions for Laboratory Analysis

Ouchterlony Lab

1. a) Explain the molecular events responsible for the formation of a precipitin band.
   b) Is there any situation in which antibody and antigen molecules may encounter one another without generating a precipitin band?
2. What is meant by the equivalence zone and how is this area detected in an Ouchterlony test?
3. How would the appearance of the precipitin band be expected to change if antigen levels are in excess of antibody?
4. What might cause two or more precipitin bands to form?
5. Attach a labeled sketch of your plates.

Radial Immunodiffusion Lab

1. Explain the molecular events essential to the formation of precipitin rings. Why are the rings circular?
2. Account for the varying sizes of rings.
3. Make a small table that compares and contrasts the Ouchterlony procedure and results to the RID procedure and results.
4. Would it be possible to have such a low concentration of antibody in the agar or antigen in the well that precipitin rings are not observed?

Appendix D3 (continued)

Student Questions for Laboratory Analysis

Immunoelectrophoresis (Edvotek, 1998)

1. Describe the function of the blue dye added to the protein solutions undergoing electrophoresis.
2. Explain why the precipitin bands take on the form of an arc?
3. Compare and contrast the procedure and results of immunodiffusion and immunoelectrophoresis.
4. Provide a sketch of your precipitin arcs bordering the whole serum sample. What does each arc represent?
5. Predict the resulting appearance of immersing the final gel block in a protein stain.

Western Blot Simulation of HIV Detection:

1. Why is it important to transfer the separated proteins to a membrane for immunological detection?
2. Consider the properties of proteins and agarose. Would higher or lower molecular weight proteins be more readily transferred? Would a block with greater or lesser agarose concentration assist enhance transfer?
3. Define the function of the positive and negative controls.
4. Differentiate this Western Blot from the earlier studied Southern Blot (genetics).
5. Attach your graph to the questions and circle the estimated molecular weight.

ELISA Systemic Lupus Erythematosus Lab Analysis Questions

1. Outline the procedural steps taken in preparing final spot-plate results. Begin with whole blood samples.
2. Indicate the meaning of a positive and negative test in terms of the presence or absence of a specific molecule.
3. Describe the possible symptoms in a patient who possesses the molecule mentioned in question two.
4. Make a series of sketches that show the molecular involvement and changes occuring in a positive test. Label each component of your sketches or provide a labeled Key to your sketches.

Appendix D4

Wampole Pregnancy Test Lab

Procedure

1. Obtain 1.0 mL of each of the three mock urine samples from the central supply table. Correctly label your samples.

2. Obtain three microscope slides and label each in the corner with the same letter as the urine sample to be used on the slide.

3. Place one drop (using the pipet within the reagent bottle) of antibody reagent (anti-HCG) in the center of each of the slides.

4. Using the appropriate "urine" samples and three separate plastic disposable pipets, transfer two drops of sample to the antibody on the corresponding slide.

5. Place one drop of latex reagent (central supply table) next to, but not touching, each antibody-urine mixture.

6. Using three separate toothpicks, stir the antibody-urine drop into the latex reagent, spread the mixture into a circular area about the size of a nickel.

7. After mixing the reagents, rock each slide gently back and forth ten times per minute.

8. After two minutes of contact time, read the slide for presence or absence of agglutination and determine whether each sample is from a pregnant or non-pregnant female.

9. Call your instructor to explain and verify your results.

Rationale

The antibody used is anti-HCG produced by monoclonal technology using a mouse-myeloma hybridoma. Anti-HCG will naturally bind with the hormone of pregnancy, HCG. Latex micro spheres are coated with HCG molecules. When anti-HCG contacts urine with no HCG (negative), the HCG molecules remain in a free state and are capable of binding to the latex spheres causing agglutination of the spheres. In positive urines, the HCG of the urine binds to the anti-HCG blocking its ability to bind with the HCG-laced latex spheres. No agglutination indicates a positive test.

Appendix E1

IMMUNOLOGY PRETEST.

Name _____

Multiple Choice. Select the best answer.

1. Which of the following is not an immune system component organ?
   a) spleen b) thyroid c) thymus d) tonsils
   e) bone marrow

2. Which of the following is incorrectly paired with respect to it's function?
   a) plasma cell-antibody synthesis
   b) helper T cell-lysis of Cancer cells
   c) cytoxic T cells-perforin release
   d) macrophage-antigen presentation
   e) memory B cell-rapid proliferation when encountering specific antigen

3. The final lymph conducting channel returning fluid to the bloodstream
   a) lymph capillaries b) lymph vessels
   c) lymph nodes d) thoracic duct e) subclavian veins

4. Humoral immunity is a function of:
   a) B-lymphocytes b) Helper T Lymphocytes
   c) Macrophages d) Natural Killer Cells
   e) all of the above

5. The word "immunoglobulin" is another word for:
   a) antigen b) blood type c) antibody
   d) interferon e) human leukocyte antigens

6. CD4 markers are associated with:
   a) cytoxic T cells b) suppressor T cells
   c) helper T cells d) all of the previous selections
   e) only a and c

7. Complement activation: a) stimulates inflammation
   b) attracts phagocytes c) enhances phagocytosis
   d) induce lysis e) all of the above

8. Newborn infants gain the greatest amount of early immunity from:
   a) very early immunizations
   b) contact with environmental bacteria and viruses
   c) contact with family members
   d) antibodies received across mom's placenta
   e) commercial feeding formulas

Page Two

9. The body's largest single mass of lymphoid tissue:
   a) tonsils b) adenoids c) bone marrow
   d) spleen e) thymus

10. HIV selectively infects the immune cells known as:
   a) the B cell b) the cytoxic T cell
   c) the helper T cell d) the memory B cell
   e) Monocytes

11. A vaccination will induce:
   a) naturally acquired passive immunity
   b) naturally acquired active immunity
   c) artificially acquired passive immunity
   d) artificially acquired active immunity
   e) all of the above

12. The naturally occuring antiviral proteins:
   a) complement b) interferons c) interleukins
   d) immunoglobulins e) tumor necrosis factors

13. Graft versus host response is most logically associated with:
   a) bone marrow transplantation
   b) kidney transplantation
   c) heart valve transplantation, pig-to-human
   d) skin grafting e) liver transplantation

14. Recognition of <u>tumor specific antigens</u> is a function most closely associated with:
   a) natural killer cells b) cytoxic T cells
   c) B lymphocytes d) plasma cells e) macrophages

15. The proper chronology of T-cell differentiation:
   a) hemocytoblast----bone marrow----lymphoid stem cell---thymus
   b) hemocytoblast---bone marrow----lymphoid stem cell----bone marrow
   c) hemocytoblast---bone marrow----lymphoid stem cell----thyroid
   d) hemocytoblast---bone marrow---lymphoid stem cell----spleen
   e) hemocytoblast---thymus---lymphoid stem cell---bone--marrow

16. Monoclonal antibodies are:
   a) produced from clones of memory cells
   b) used to produce large quantities of interferon
   c) produced by cultures of hybridoma cells
   d) produced by clones of T-cells fused with tumor cells
   e) produced by recombinant DNA methods

Page Three

17. The body produces antibodies complementary to foreign antigens. The process by which the body comes up with the correct antibodies specific to a given antigen is most like which of these analogies?
    a) going to a dress maker and having a dress made to fit you.
    b) ordering the lunch special at a restaurant without looking at the menu
    c) going to a shoe store and trying on many pairs until you find the perfect fit
    d) picking the first video that you have not yet seen
    e) select a winning lottery ticket by means of a random drawing

18. Which of the following could prevent the appearance of the symptoms of an allergy attack?
    a) blocking the attachment of IgE antibodies to the mast cells
    b) blocking the antigenic determinants of the IgM antibodies
    c) reducing the number of T helper cells in the body
    d) only a and b are correct
    e) only b and c are correct

19. The clonal selection theory implies that :
    a) related people have similar immune responses
    b) only certain cells can produce interferon
    c) memory cells are present at birth
    d) the body selects which antigens it will respond to
    e) antigens activate specific lymphocytes

20. A person suffering from AIDS would be unlikely to suffer from which of the following?
    a) cancer b) rheumatoid arthritis c) hepatitis
    d) tuberculosis e) influenza

Page Four

Part Two. Fill-in the blank with appropriate word or phrase.

1. _____ name for final cell type producing monoclonal antibodies.
2. _____ tissue markers associated with antigen presenting cells like macrophages
3. _____ Term used to describe reaction of cellular antigens combining with complimentary antibodies.
4. _____ The movement of phagocytes in or out of capillaries is known as:
5. _____ systemic mast cell activation by an allergen results in _____.
6. _____ The acronymn ELISA means:
   _____
   _____
7. _____ Collective name for thymus hormones.
8. _____ Natural Killer cells release ____ on their targets.
9. _____ Fever inducing proteins released by macrophages
10. _____ T-cells are responsible for _____ -mediated immunity.

Part Three. Label the following Immune System Structures.
(See diagram on next page)

1. _____ 2. _____
3. _____ 4. _____
5. _____ 6. _____
7. 8.

Page Five

Part Four. In a sentence, describe a function specific to each label in the previous diagram.

1.

2.

3.

4.

5.

6.

7.

8.

Part Five. Short Answer Responses.

1. Use a lableled sketch to show the basic 2-dimensional structure of a typical antibody.

2. Use a sketch accompanied by explanation, explaining the activation of a specific B-lymphocyte.

3. List or describe four specific actions of antibodies resulting from their combination with complimentary antigens.

Appendix E2

IMMUNOLOGY POST TEST.

Name _____

Multiple Choice. Select the best answer.

1. The purpose of an enzyme in any ELISA test is:
   a) to activate a specific monoclonal antibody
   b) opsonization
   c) to induce a precipitation reaction in a positive identification
   d) to induce a color change in a positive identification
   e) to catalyze antibody-antigen binding in a positive test

2. Which of the following is not an immune system component organ?
   a) spleen b) thyroid c) thymus d) tonsils
   e) bone marrow

3. Which of the following is incorrectly paired with respect to it's function?
   a) plasma cell-antibody synthesis
   b) helper T cell-lysis of Cancer cells
   c) cytoxic T cells-lymphotoxin release
   d) macrophage-antigen presentation
   e) memory B cell-rapid proliferation when encountering specific antigen

4. The primary chemical released by mast cells in an anaphylactic allergic response:
   a) heparin b) prostaglandins c) histamine
   d) immunoglobulins e) epinephrine

5. The final lymph conducting channel returning fluid to the bloodstream
   a) lymph capillaries b) lymph vessels
   c) lymph nodes d) thoracic duct e) subclavian veins

6. Which of the following could prevent the appearance of the symptoms of an allergic attack?
   a) blocking the attachment of IgE antibodies to mast cells
   b) blocking the antigenic determinants of the IgM antibodies
   c) reducing the number of helper T cells
   d) only a and b are correct
   e) only b and c are correct

Page Two-Post Test

7. Which of the following would be most effective in treating an individual who has been bitten by a poisonous snake emitting fast acting toxin?
   a) vaccination with a weakened from of the toxin
   b) injections of antibodies to the toxin
   c) injection of interleukin I
   d) injection of interleukin II
   e) injection of interferon

8. Humoral immunity is a function of:
   a) B-lymphocytes b) Helper T Lymphocytes
   c) Macrophages d) Natural Killer Cells
   e) all of the above

9. In mammalian defenses all of the following are considered nonspecific except:
   a) the B and T lymphocyte action b) the skin
   c) mucous membranes d) the inflammmatory response
   e) antimicrobial proteins

10. The word "immunoglobulin" is another word for:
   a) antigen b) blood type c) antibody
   d) interferon e) human leukocyte antigens

11. The following events occur when a mammalian immune system first encounters a pathogen. Place them is a correct sequence, and then choose the answer that indicates the correct sequence.
   I. pathogen is destroyed
   II. lymphocytes secrete antibodies
   III. antigenic determinants from pathogens bind to antigen receptors on lymphocytes
   IV. lymphocytes specific to antigenic determinants from pathogen become numerous
   V. Only memory cells remain.

   a) I, III, II, IV, V b) III, II, I, V, IV
   c) II, I, IV, III, V d) IV, II, III, I, V
   e) III, IV, II, I, V

12. CD4 markers are associated with:
   a) cytoxic T cells b) suppressor T cells
   c) helper T cells d) all of the previous selections
   e) only a and c

13. Complement activation: a) stimulates inflammation
   b) attracts phagocytes c) enhances phagocytosis
   d) induce lysis e) all of the above

Page Three-Post Test

14. Newborn infants gain the greatest amount of early immunity from:
    a) very early immunizations
    b) contact with environmental bacteria and viruses
    c) contact with family members
    d) antibodies received across mom's placenta
    e) commercial feeding formulas

15-19. Match the following answers with the phrase that best describes them. Answers may be used one or more times or not at all.
    a) cytoxic T cells b) delayed hypersensitivity
    c) helper T cells d) T suppressor cells
    e) B cells

15. Form plasma cells that give rise to antibodies
16. Release cytokines that activate B cells
17. Release perforin causing target cells to lose cytoplasm
18. cooperate with macrophages and then interact with effector cells causing them to make antibody
19. attack and destroy intracellular pathogens such as the tuberculosis bacterium

20. The body's largest single mass of lymphoid tissue:
    a) tonsils b) adenoids c) bone marrow
    d) spleen e) thymus

21. HIV selectively infects the immune cells known as:
    a) the B cell b) the cytoxic T cell
    c) the helper T cell d) the memory B cell
    e) Monocytes

22. A vaccination will induce:
    a) naturally acquired passive immunity
    b) naturally acquired active immunity
    c) artificially acquired passive immunity
    d) artificially acquired active immunity
    e) all of the above

23. The MHC complex is important in:
    a) distinguishing self from nonself
    b) recognizing parasitic protozoans
    c) identifying bacterial pathogens
    d) identifying abnormal cells
    e) both a and d are correct

Page Four

24. The naturally occuring antiviral proteins:
    a) complement b) interferons c) interleukins
    d) immunoglobulins e) tumor necrosis factors

25. Graft versus host response is most logically associated with:
    a) bone marrow transplantation
    b) kidney transplantation
    c) heart valve transplantation, pig-to-human
    d) skin grafting e) liver transplantation

26. Why can the immune response be described as polyclonal?
    a) blood contains many different antibodies to many different antigens
    b) construction of a hybridoma requires multiple types of cells
    c) multiuple immunoglobulins are produced from descendants of a single cell type
    d) diverse antibodies are produced fro different epitopes of a specific antigen
    e) macrophages, T cells, and B cells are involved in a normal immune response

27. Recognition of <u>tumor specific antigens</u> is a function most closely associated with:
    a) natural killer cells b) cytoxic T cells
    c) B lymphocytes d) plasma cells e) macrophages

28. The proper chronology of T-cell differentiation:
    a) hemocytoblast----bone marrow----lymphoid stem cell---thymus
    b) hemocytoblast---bone marrow----lymphoid stem cell----bone marrow
    c) hemocytoblast---bone marrow----lymphoid stem cell----thyroid
    d) hemocytoblast---bone marrow---lymphoid stem cell-----spleen
    e) hemocytoblast---thymus---lymphoid stem cell---bone--marrow

29. Monoclonal antibodies are:
    a) produced from clones of memory cells
    b) used to produce large quantities of interferon
    c) produced by cultures of hybridoma cells
    d) produced by clones of T-cells fused with tumor cells
    e) produced by recombinant DNA methods

Page Five

30. The body produces antibodies complementary to foreign antigens. The process by which the body comes up with the correct antibodies specific to a given antigen is most like which of these analogies?
    a) going to a dress maker and having a dress made to fit you.
    b) ordering the lunch special at a restaurant without looking at the menu
    c) going to a shoe store and trying on many pairs until you find the perfect fit
    d) picking the first video that you have not yet seen
    e) select a winning lottery ticket by means of a random drawing

31. Which of the following could prevent the appearance of the symptoms of an allergy attack?
    a) blocking the attachment of IgE antibodies to the mast cells
    b) blocking the antigenic determinants of the IgM antibodies
    c) reducing the number of T helper cells in the body
    d) only a and b are correct
    e) only b and c are correct

32. The clonal selection theory implies that :
    a) related people have similar immune responses
    b) only certain cells can produce interferon
    c) memory cells are present at birth
    d) the body selects which antigens it will respond to
    e) antigens activate specific lymphocytes

33.. A person suffering from AIDS would be unlikely to suffer from which of the following?
    a) cancer b) rheumatoid arthritis c) hepatitis
    d) tuberculosis e) influenza

34. The conclusive test for the presence of Human Immunodeficiency Virus identifies ____ and is called a(n) ____.
    a) reverse transcriptase, electrophoresis
    b) gp-120, Western blot
    c) viral RNA, ultracentrifugation
    d) viral DNA, electrophoresis
    e) antibodies against HIV, Ouchterlony Test

Page Six.
Part Two. Fill-in the blank with appropriate word or phrase.

1. _____ name for final cell type producing monoclonal antibodies.
2. _____ full name for the lab test RID
3. _____ tissue markers associated with antigen presenting cells like macrophages
4. _____ The form of immunity induced by _____ vaccination
5. _____ Term used to describe reaction of cellular antigens combining with complimentary antibodies.
6. _____ The movement of phagocytes in or out of capillaries is known as:
7. _____ MHC
8. _____ systemic mast cell activation by an allergen results in _____.
9. _____ The acronymn ELISA means: _____ _____
10. _____ General name for the class of compounds allowing T's, B's, and macrophages to communicate
11. _____ Cell type associated with IgE's and allergic response .
12. _____ Natural Killer cells release ____ on their targets.
13. _____ Fever inducing proteins released by macrophages
14. _____ Phrase indicating rejection of recipient by transplanted tissue
15. _____ T-cells are responsible for _____ -mediated immunity.
16. _____ Enhancement of phagocytosis by antibody-complement attachment
17. _____ Specific cell type responsible for a second-set response
18. _____ Portion of an antigen interacting with the variable region of an antibody.
19. _____ Antibody rich milk secretion passed from mother's to their newborns
20. _____ Antibody class referred to as "secretory".

Page Seven. Post Test

Part Three. Label the following Immune System Structures. (See diagram on next page)

1. _____ 2. _____

3. _____ 4. _____

5. _____ 6. _____

7. _____ 8. _____

Part Four. In a sentence, describe a function specific to each label given above. Match the number of structure and function.

1.

2.

3.

4.

5.

6.

7.

8.

Part Five. Short Answer Responses.

1. a) Describe the process of clonal selection in B-cell activation.
   b) Describe the process of T-cell mediated B cell activation. Begin with an APC-T-cell combination.

2. a) Name the five classes of antibodies.
   b) Describe a functional activity unique to each class.

3. a) What are the primary immunological concerns in a kidney transplant?
   b) What are the general steps taken to insure transplant success?

4. Use a labeled illustration or a chronological listing to describe your body's immune response to pneumococcal bacteria.

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5. a) Explain the process of making a monoclonal antibody.
   b) List three specific applications of monocolonal antibodies.

6. Assign a function to each:
   a) complement b) interferon c) lysozyme
   d) normal flora

7. Describe the general role of the lymph nodes and spleen in immunology.

8. Explain how you would fight off a flu virus.

Appendix E3

Immunology Quick Quiz-Antibodies*****Good Luck!

Name_____

1. The cellular producer of immunoglobulins is

   _____

2. Where would you most likely find a functioning IgA?

   _____

3. Considering antibody structure, explain why Rh incompatibilities involving mother and unborn child were historically more frequent and serious than ABO incompatibilities.

4. Sketch and label a typical two-dimensional model of an IgG.

5. How are monoclonal antibodies produced? What are several uses of monoclonal antibodies? (ON BACK PLEASE)

Appendix E4

Rubrics For Short Answer Response Evaluation

Question 1.

- -Specific epitope of antigen
- -Contacts Ig receptor on complimentary B cell
- -Signal transduction
- -Gene Activation
- -Mitosis
- -Differentiation to Plasma Cells
- -Ig Production

Question 2.

- -Heavy polypeptide chains
- -Light polypeptide chains
- -General "Y" shape, disulfide bridges, carbo.
- -Constant regions
- -Variable regions

Questions 3.

- -Agglutination
- -Precipitation
- -Opsinization
- -Lysis