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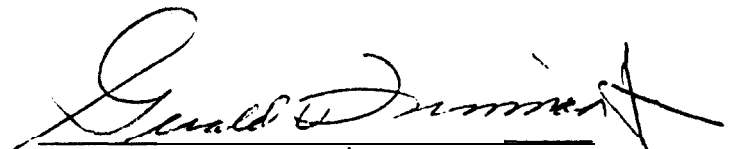
FROM: Center for Biologic8 Evaluation and **Research**,
Food and Drug Administration

SUBJECT: "Points to Consider in the Collection, Processing, and Testing of
Ex-Vivo - Activated Mononuclear **Leukocytes for Administration to**
Humans"

TO: Manufacturers of Biological Products and Other Interested Parties

These "Points" have been developed for mononuclear **leukocytes cultured and** activated *ex-vivo* for administration to patients. These "Points" are not regulations nor are they guidelines but represent the current thinking that **the Center** for Biologic8 Evaluation and Research (**CBER**) staff believe should be considered at this time.

It is our intention to continuously revise and update this document in order to improve its usefulness, effectiveness, and practicality. We invite your review and comments on these "Points". Your comments should be addressed to the Director, Division of Cytokine Biology, Center for Biologic8 Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Room 2D-20, HFB-800, Bethesda, Maryland 20892 (Telephone: 301-496-8245).


Gerald V. Quinnan, M.D.

POINTS TO CONSIDER IN **THE** COLLECTION, PROCESSING, AND
TESTING OF RX-VIVO ACTIVATED **MONONUCLEAR** LEUKOCYTES FOR
ADMINISTRATION TO HUMANS

Center for **Biologics Evaluation and** Research
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**POINTS TO CONSIDER IN THE COLLECTION, PROCESSING,
AND TESTING OF EX-VIVO - ACTIVATED MONONUCLEAR LEUKOCYTES FOR
ADMINISTRATION TO HUMANS**

I. INTRODUCTION

This document is concerned with the collection, processing, and testing of ex-vivo - activated mononuclear leukocytes intended for administration to humans. Because the development of activated mononuclear leukocytes for human use is in an early stage, approaches to establishing safety, purity, and potency are expected to evolve with advances in science and technology. It should be kept in mind that this document is not all inclusive, and that certain points will not be applicable in all situations. CBER staff have been reviewing protocols for the production and administration of activated mononuclear leukocytes as part of applications for IND exemptions for several years. Based upon that experience, CBER staff feel that the points discussed below will provide useful guidance to manufacturers engaged in the production of activated mononuclear leukocyte products. CBER staff generally expect such manufacturers to consider these points when filing for an IND exemption; additionally, the manufacturer should be familiar with the current regulations, guidelines, and "Points to Consider" documents, which may be obtained from the Biologics Freedom of Information Staff, 301-496-9508 (Biologics FOI).

II. CELL COLLECTION AND SEPARATION

A. COLLECTION PROCEDURES

The technique for cell collection should be specified; e.g., phlebotomy, lymph collection, cytopheresis, surgery. The location, facilities, equipment,

standard operating **procedures, precautions** against contamination by micro-organisms **and** toxins, and other details of the method **of obtaining cells** to be activated **should be described**. Specifying that **cells are to be** collected by cytopheresis **according** to the standards for approved cellular products in a registered blood bank would substitute for **these facilities and** methods descriptions.

B. RISKS OF COLLECTION

If cells are to be collected by a technique which is of greater risk than venipuncture and which is not otherwise medically **indicated, then the risk of** the collection procedure should be justified. Such justification would generally include both demonstration of the feasibility **of** the proposed cell collection and activation, and development of scientific data supporting the possibility of clinical benefit.

C. ALLOGENEIC DONORS

Collection of cells from donors other than the intended recipient should usually meet the standards for collection of licensed source leukocytes. Donors of allogeneic cells should usually meet the standards for blood donors (21 CFR 640.3). Any deviation from these standards should **be** justified. When allogeneic cells are used, the extent of cross-matching should be specified (**e.g.**, ABO blood group, major HLA loci, etc.). In addition, the details of the typing schema and criteria for an adequate match should be specified. In general, to reduce the risk of transmission of adventitious agents, patients

should not receive cells from **more than one donor**. **Due to concerns about both cell rejection and graft-versus-host reactions, allogeneic activated lymphocytes should be used only in cases where the use of autologous cells is not feasible or is demonstrably inferior.**

D. CELL SEPARATOR PROCEDURES

Techniques, reagents, and equipment used for cell separation should be **described in detail**. When **monoclonal** antibodies **are to be used**, the "Points to Consider in the **Manufacture of Monoclonal Antibody Products for Human Use**, (1987)" should be consulted.

E. CELL TESTING

At appropriate steps in the cell collection and separation **process, each** lot of cells should be tested for the **purpose** of quality control. **(A lot is defined as the cells from a single collection from a single donor.)** Depending upon the nature of **the cell collection and separation, appropriate parameters** may include yield of collection and separation, viability, phenotype, and functional capacity. Techniques, reagents, and equipment used **for** cell testing should be **described in detail**. Acceptance criteria **should be** developed.

III. COMPONENTS OF THE CULTURE MEDIUM

A. GENERAL CONSIDERATIONS

All components should be of sufficient purity (e.g., water in contact with

the cells should meet the standards for water for injection) that residual impurities do not have significant impact on the cultured cells or the recipient. Each laboratory culturing mononuclear leukocytes should ensure that the quality of all components used in the culture is evaluated and that specifications are established. If the medium, with or without serum, is from a commercial source, that manufacturer may file a Raster File with the Center for Biologics Evaluation and Research to describe the components of the medium, and authorized cross-reference to that master file may be used. Attempts should be made to establish the necessity for each component used in the culture and to eliminate those components not required.

B. USE OF SERUM

The use of any serum should be avoided unless it can be shown that serum is required for cell activation. If serum must be used, the following points should be considered:

1. Adventitious Agents

The risk of transmission of adventitious agents by human serum may be decreased as follows:

Donor Screening - If allogeneic serum must be used, every donation to the serum pool should be non-reactive for HBsAg, HIV-1, and HTLV-1 antibody by licensed tests, and the serum vendor should be qualified to assure aseptic handling of the serum. If pooled serum

is to be used, it should come from the smallest feasible number of donors. The risk of contamination might be further reduced by using serum from pedigreed donors only and storing serum for six months prior to use. During this time period, the *donors* should be monitored for good health, absence of serological evidence of infection with HIV-1 and hepatitis B, and absence of liver enzyme elevations in serum.

Serum Pool Testing - Consideration should be given to testing serum pools for retroviruses and other potential pathogens by appropriate culturing techniques.

Detection and Investigation of Possible Contamination - Recipients of cells cultured with allogeneic serum should be monitored *for* evidence of hepatitis, mononucleosis, or other viral illnesses. If such illnesses do occur, the pooled serum should be investigated as a possible source. For this reason, aliquots of individual serum donations and pooled serum should be retained for at least one year after the administration of cells cultured in the presence of that serum.

Sterilization of Serum - Serum should be treated in a manner which inactivates a broad range of potential viral contaminants unless it is found that such treated serum cannot support cell activation. Additionally, the serum should be sterilized by passage through washed filters before it is added to the culture medium.

2. Plasma Source

Serum from Source Plasma is preferable to that from recovered plasma. Laboratory salvage plasma is unacceptable for use in manufacture of products intended for parenteral administration. For guidance in the use of defibrinated source plasma, the regulations regarding Source Plasma (21 CFR 640, subpart G) should be consulted. In addition, the defibrination procedure should be described and quality control procedures provided to ensure the completeness of removal of clotting factors, purity, and sterility.

3. Serum Efficacy

It is suggested that each serum lot be tested for its ability to support cell activation, as some lots appear to interfere with some types of leukocyte activation.

4. Animal Serum

If other than human serum is to be used, each lot of serum should be screened by appropriate tests for the presence of potential adventitious agents, including human pathogens. For example, bovine serum should be screened for viral and mycoplasma contamination. Procedures for the inactivation of potential pathogens, such as heat and irradiation, should be considered. Recipients of cells grown in heterologous serum should be tested for development of heterologous immune responses.

C. USE OF HUMAN BLOOD COMPONENTS

If the medium contains human blood components such as albumin and transferrin, these components should be fully described with respect to source (e.g., albumin should be licensed) and purity, including description of procedures used to screen for, remove, or inactivate adventitious agents. Validation of such procedures should be provided.

D. USE OF CONDITIONED MEDIUM

The use of conditioned medium from cell cultures in the process of mononuclear leukocyte activation presents additional concerns regarding risks and consistency. Efforts to establish the requirement for conditioned medium in cell activation, to determine the essential ingredient(s) of the conditioned medium, and to replace conditioned medium with defined reagents, are strongly encouraged. When conditioned medium is required the following points should be considered:

- . The conditioned medium is subject to all the considerations listed above in sections A, B, and C.**
- . The source, processing, and description of the conditioned medium should be provided in detail. In addition, the source of the cells used to condition the medium should be described.**
- . When cells from human donors are used in the production of conditioned medium, the donor should be examined for freedom from**

infectious agents. It is suggested that the donors of the cells for the conditioned medium and the recipients of the activated mononuclear leukocytes made in conditioned medium be followed by hepatic *enzyme* tests and serological tests for exposure to HTLV-I, HIV-I, hepatitis A, hepatitis B, CMV, and BBV.

- **When cell lines are used in the production of conditioned medium, the “Points to Consider in the Characterization of Cell Lines to Produce Biological Products, 1987” (available from Biologics FOI) should be consulted.**
- **The use of autologous cells to condition the medium would reduce the risk of transmission of viral diseases; however, the potential for the ex-vivo amplification of viruses should still be considered.**

E. USE OF ANTIBIOTICS

Beta-lactam antibiotics should not be used in the culture medium. If antibiotics are used, then the cells should be labeled as processed with such antibiotics and they should not be used in patients with known hypersensitivity to these drugs. In addition, model cultures should be set up without antibiotics to demonstrate the ability to maintain sterility.

F. OTHER CULTURE COMPONENTS

All mitogens, cytokines, growth factors, and other agents or chemicals used in cell activation and culture should be fully described. If the

manufacturer **of** these **components-** has **filed a** Waster File or **IND** with the Center **for Biologic8 Evaluation and Research for a particular** component, **authorized cross-reference to** the **file may be made.** **When monoclonal** antibodies are to be used., the "Points to Consider in the Manufacture of Xonoclonal Antibody Products for Human Use, (1987)".

G. **TESTING OF FORMULATED MEDIUM**

Each final lot **of** completely formulated medium (i.e., with serum and growth supplements, where applicable¹ should be tested for sterility and for its ability to activate and/or support the growth of ex-vivo cultured cells intended for administration to patients. Acceptance criteria should be developed.

IV. CELL CULTURE

A. FACILITIES **AND** PERSONNEL

Guidance regarding good manufacturing practice with regard to facilities and personnel may be obtained by consulting 21 CFR 210, 211, and 606. Because cell activation cultures generally provide a good substrate for microbial growth and live cells will not tolerate many forms of decontamination, contamination of the cells is a particular concern. The following procedures regarding facilities and personnel may be **of value** in limiting the extent of this problem. Insofar as possible, the facilities should be dedicated solely to these cultures; at a minimum, the incubators and hoods should be restricted to activated cell cultures. Validated decontamination procedures should be

employed between processing of individual lots of cells. Microbiological agents and cells which could potentially infect or contaminate the cell cultures should not be present in the facility. The facility should be restricted to authorized personnel only.

The cell culture and testing should be continuously supervised by personnel qualified in mammalian cell culture techniques. A training program for new personnel and a retraining program for experienced personnel should be in place. Personnel who have previously worked that day with microbiological agents and/or cells which could potentially infect or contaminate the cell cultures should not work with the cells. In addition, persons whose presence could adversely effect the safety and purity of the activated cells should be excluded from the processing facility.

B. PROCEDURES

Summary protocols for cell culture , washing, and formulation techniques should be described. Detailed standard operating procedures should be maintained in the laboratory and updated upon any change in procedure. Aliquots of cells and media at harvest should be reserved for later testing. If the cell culture technique is modified, the impact of the change on the yield, viability, function, and purity of the cells should be assessed. When an animal or in-vitro efficacy model is available, major changes in procedures should be validated in such models for effects on the activity of the cell preparation.

The quality control systems which ensure against misidentification and cross-contamination **of** cell lots should be described, and should **be** applied to longer term cultures **periodically and to all** cultures **at the end of the** in-vitro period before infusion.

C. **NEW EQUIPMENT**

The use of automated cell culture equipment, including bioreactors and closed or semiclosed bag systems, may be valuable if it minimizes human handling of the cultures and thereby reduces opportunities for contamination. For any such culture system to be used: **(1)** a description of the system should be provided in detail; **(2)** data should be presented which describe the viability, function, and yield of cells produced; **(3)** all components should be shown to be non-toxic; and, **(4)** evidence should be presented that sterility can be maintained routinely. The cell culture equipment may be subject to regulations under the device authorities, i.e., **510K, premarketing approval (PMA)**.

D. **QUALITY CONTROL**

Quality control procedures should be in effect to qualify all **the** components, including glass and plastic ware, used in the separation, culture, and final processing of the cells. The components should be examined for identity and likely contaminants. Bioactive components should also be tested for function. Lot records should be maintained which identify all steps in the preparation of each lot of cells. These records should indicate the lot **number of every component and all** test results.

V. TESTING AND QUALITY CONTROL OF THE
ACTIVATED MONONUCLEAR LEUKOCYTES

Testing of lots **of activated mononuclear leukocytes includes those tests done** on the first several lots of cells (**and** after any process change) to **validate the** process, **as well as those tests done on every lot to determine safety and provide information. Specifications for the acceptability of lots of cells should** be developed **on the basis of actual experience.**

A. TESTS FOR EVERY LOT

Testing on every lot of activated mononuclear leukocytes **should include** the following:

1. Yield and Viability

Yield and viability **should** be **assessed** prior to infusion; and if **the** cells are cultured for longer than a few days, yield and viability should be **assessed** periodically during the culture. **Low viability** suggests poor process control or **lack** of consistency, and might also indicate the presence of toxins or other contaminants. Therefore, reprocessing and/or administration of cells with low viability may introduce safety risks in addition to the direct risks of administering dead cells.

2. Identification of Source

Cell lots should be identified by donor source and source leukocyte lot number, if appropriate.

3. Sterility

Every lot of cultured cells should be tested for sterility before the cells are reinfused into the patient. It is suggested that aliquots of the culture medium be taken at regular intervals beginning three to four days after initiation of the culture and including a sample taken approximately 24 hours before infusion of cells into the patient. Guidance for the detection of microbial contaminants may be found in 21 CFR 610.12.

If antibiotics were used in the culture, care should be taken to dilute the culture medium when the sterility tests are initiated so that the antibiotics do not interfere with growth of any contaminating microorganisms. The sterility test method used should be validated. The microbial culture(s) should be checked just before infusion of cells into the patient, and should also be maintained for the full culture period specified. It is recommended that an additional check for contamination be made just before cell infusion using acridine orange stain or Gram stain of the culture fluid and/or its sediment. Cultures maintained for longer than a few days should be screened for mycoplasma (see "Recommended Test Procedures for Mycoplasmas" which is available from Biologics FOI).

4. Purity

- Malignant cells - If the cells were isolated from tissues containing malignant cells, the absence of malignant cells should

be documented **by techniques of described sensitivities,**

Administration of activated mononuclear leukocytes contaminated with autologous malignant cells to individuals with cancer presents theoretical risks, since the malignant cells may be presented in a manner and location substantially different from endogenous malignancy and since ex-vivo activation procedures might select for relatively "immunoresistant" malignant cells. Testing for the kinetics of malignant cell depletion in activation cultures should be considered.

- **Transformed cells - Cells cultured through many passages may transform to an exogenous growth factor-independent phenotype. While the potential for such cells to be malignant after reinfusion is unknown, it is felt that the intentional reinfusion of such cells into humans would need to be supported by extensive preclinical evaluation. If the activated mononuclear leukocytes are growth factor dependent (e.g., IL-2), and cultured more than two weeks, consideration should be given to culturing an aliquot of the cells in the absence of growth factors at a suitable period prior to infusion to test for factor-independent growth of malignant cells or transformed leukocytes.**
- **Contaminating cells - For mononuclear leukocytes cultured through several passages, tests should be instituted to ensure the absence of contamination with or replacement by other types of cells (e.g., cells from other donors or cell lines).**

- Lymphocytes **targeted against the recipient** - When allogeneic cells **are used, they may have cytotoxic activity or other activities with specificity for normal cells** **the intended recipient. Where this concern is relevant, cells should be tested** prior to administration for activity **specific for normal cells of the intended recipient and the recipient should be monitored for graft-versus-host type reactions.**
- **Other impurities** - Components used in activation **and** culture which **are not intended for** use in humans (e.g., phorbol esters) should be measured in the final product. In some cases e.g., residual bovine serum proteins, calculation of potential residual levels may be substituted for measurement.

5. Potency

If the mononuclear leukocytes **have been activated to become** cytotoxic, the cytotoxic activity of **each** lot of these cells should be demonstrated . This **may be done** using established cell lines or primary tumor cells **as** targets. **Because it may be difficult to** compare results among different laboratories, a program of reagent exchange among quality control personnel at the different laboratories is suggested. Cells activated with respect to other parameters should be tested with an appropriate assay system for the relevant induced activity (e.g., antigen presentation, helper function, suppressor function, or lymphokine release). If the activation parameter which

best correlates with potency is unknown, the better the cells are characterized the greater the opportunity to determine relevant measures **of** potency.

B. TESTS FOR **REPRESENTATIVE** LOTS

Some tests **may** need to be done only on the first several lots of activated mononuclear leukocytes **and** following any process change. The results **of** such tests provide **information** on the cultures as well as document the consistency of the procedures. Some tests for which representative lot testing may suffice include the following:

1. Detailed Characterization

Characterization of cultured cell types to a greater extent than would be done on a lot-to-lot basis. Such characterization might include the measurement of phenotype distribution by **IFA** slide testing or flow cytometry.

2. Tests of Cytokine Production

Tests for production by the cells of vasoactive factors, immunomodulating factors, factors which modulate hematopoiesis growth factors, differentiation factors, known characterized cytokines, etc.

3. Tests for Endotoxin

Limulus amebocyte lysate testing (the guideline is available from

Biologics FOI) may be appropriate if the activated mononuclear **leukocytes do not interfere with the test and** if **spiking experiments demonstrate** that endotoxin **does** not escape detection **by** binding to the cells. However, animal pyrogenicity testing as described in 21 CFR 610.13 **(b)** is suggested on pilot lots, **as well as for periodic testing** during production.

4. Tests of Clonality

In lots of lymphocytes cultured for more than three **weeks**, determination of **the** extent of clonality of the cells by studying receptor gene rearrangements **should** be considered as a means of product characterization.

VI. MONITORING RECIPIENTS OF ACTIVATED CELLS

Recipients of activated mononuclear leukocytes should be monitored clinically for bacteremia or fungemia and for viral illnesses (see section **III.B.1**). Some centers have reported a 25% infection rate with administration of activated mononuclear leukocytes. When infection occurs, the source of the infection (e.g., contaminated culture, catheter-related sepsis) should **be** determined. All incidents of contamination of cultures and/or infection of recipients should be investigated immediately and reported to the FDA.