Idiopathic Interstitial Pulmonary Fibrosis*

Contribution of Bronchoalveolar Lavage Analysis

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The interstitial lung diseases are a heterogeneous group of diseases that affect the supporting structure of the air-exchange units, especially the perialveolar tissue and alveolar walls. The initial host response to the disease process is inflammation in the alveolar walls and airspaces, causing an acute phase of mural and luminal alveolitis, followed by the consequences of prolonged or unbridled inflammation, which subsequently involve adjacent portions of the interstitium and vasculature. Eventually, interstitial fibrosis can produce scarring and distortion of lung tissue that lead to significant derangement of gas exchange function and ventilation.

As this is a diverse group of diseases, providing a satisfactory classification has been difficult. A reasonable approach is to separate broadly the interstitial lung diseases into two groups, those with known causes and those with unknown causes; each can be subclassified further according to the presence or absence of granuloma in interstitial or vascular areas as viewed in histologic specimens.

Interstitial lung diseases of known cause include several major subcategories. Occupational and environmental inhalant exposures include diseases due to inhalation of inorganic dusts, organic dusts (hypersensitivity pneumonitis), gases, fumes, vapors, and aerosols. Others include lung diseases caused by drugs and irradiation. The number of diseases with an unknown cause is likewise very large. The major subgroups within this category are idiopathic pulmonary fibrosis (IPF), or cryptogenic fibrosing alveolitis, and connective tissue (collagen-vascular) disorders with associated interstitial lung disease. Among the granulomatous diseases of unknown etiology, sarcoidosis and eosinophilic granuloma are prominent.

This review will deal only with IPF. This entity is often considered the wastebasket diagnosis or a diagnosis of exclusion after known causes of interstitial lung diseases or that associated with collagen vascular diseases have been reasonably excluded by the patient's evaluation. Yet, IPF is among the most frequent diagnoses entertained or actually made in the nongranulomatous interstitial fibrosis category, and this disease is distinctive in its own right. We will review some of its background, after which emphasis will be on the contribution of bronchoalveolar lavage (BAL) fluid analysis to current understanding of this disease process. Three general topics will be reviewed: (1) usefulness of BAL fluid analysis in diagnosis and perhaps monitoring the clinical course of the disease; (2) accuracy of intraluminal cellular findings to mirror alveolar mural and interstitial inflammatory changes; and (3) immunologic changes that have helped with pathogenetic concepts of this disease(s) of still unknown cause.

Awareness of IPF was rekindled about 50 years ago, when Hamman and Rich described a fulminating, fatal form of so-called alveolar-capillary block syndrome in which proliferating fibrous tissue virtually occluded the peripheral airways. Although interstitial pulmonary fibrosis often associated with chronic infection (tuberculosis) was well recognized, this description of an acute fibrotic disease leading to the patient's demise within a few months, unrelated to obvious infection, was new. Some skepticism was expressed after the oral presentation of these initial cases relating to the exclusion of an infectious cause, the rarity of the problem, and possible resemblance of the histologic alveolar changes to those in a canine model with influenza infection (raised in the context of another case.)

To the authors' surprise, they waited about 10 years to observe another case and then re-presented their findings with some modifications in what is usually considered the definitive description of the disease.

Subsequent evolution of the disease and the controversy about its pathologic description affirm the difficulty in characterizing a disease with no etiology and from only pathologic material. Liebow and colleagues described a variation of diffuse chronic interstitial pneumonia that they named "desquamative interstitial pneumonia" (DIP). Distinctive histologic features in lung biopsy specimens from their 18 patients were slight thickening only of the alveolar septae; accumulation of masses of large alveolar cells (considered then to be granular pneumocytes) within alveolar spaces, evidently proliferating (as many as 0.4 of these cells were in mitosis); absence of necrosis and hyaline membranes; and little loss of alveolar substance, with general preservation of the fabric of lung tissue. The "cytorrhea" of the lung was striking for the presence of desquamative, proliferating alveolar cells but small numbers of lymphocytes, and a few eosinophils and PMNs could be found. These patients had respiratory symptoms on average about 22 months; most patients were between 35 and 35 years of age. Lung histologic findings were clearly different from those seen with "usual" forms of interstitial pneumonia in which were found necrosis and intra-alveolar fibrin, irregular thickening of alveolar walls with fibrous tissue and inflammatory infiltrate, foci of increased muscle and connective tissue, loss of alveolar structure, and formation of cystic spaces. The patients with DIP seemed to have more of a superficial intra-alveolar or luminal cellular reaction; their clinical response to corticosteroid therapy was recognized to be good.

Scadding and Hinson challenged the contention of Liebow and colleagues that patients with DIP had such a distinct variant of the usual forms of interstitial pneumonia. Their definition of acute or chronic diffuse interstitial fibrosis of the lungs was presented as "diffuse fibrosing alveolitis," which allowed a flexible, and perhaps more realistic, interpretation of the lung tissue findings. The essential features of the histology were inflammation of the lung beyond the terminal bronchioles in which cellular thickening of alveolar walls was prominent, a tendency to progress to fibrosis; and large mononuclear cells, presumably of alveolar origin, within alveolar spaces. Implicit was the notion that the proportion of lung with alveolar mural thickening and fibrosis and with

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intra-alveolar cellularity might vary in different cases and represent different stages of the same case. Among the 18 patients and lung biopsy specimens analyzed, 8 were remarkable for uniformity of the lesion and slightness of alveolar wall thickening; generally, alveolar cells of granular type were more numerous. In the others alveolar wall thickness was prominent, uniform changes were not present, and alveolar cells tended to be sparse and phagocytic. The patients who were more responsive to corticosteroid therapy had the former kind of minimal alveolar wall pathologic findings, but other radiographic and clinical correlations with lung pathology were not striking. Thus, results of this study did not corroborate such a distinctive patient group with DIP histology, but included instead these patients within the wide spectrum of interstitial pneumonia. Patients selected at a time when the extent of alveolar wall destruction was not severe might be expected to do better. The practical implication was to recognize the predominantly desquamative type of fibrosing alveolitis and the mural type as variations within the range of pathologic changes.

Whether patients with histologic criteria of DIP really represent a unique subset of patients or are only in an earlier phase in a continuing disease process is controversial and still conjectural. It seems evident that many investigators subsequently have adopted the view that in affected lung tissue, histologic changes can be observed that have features of both DIP and usual interstitial pneumonitis (UIP). They are part of an evolving pattern of lung injury and tissue response that may exist within the same biopsy specimen. Desquamated alveolar cells have been identified now as principally macrophages. Many of the recent studies that have contrasted alveolar space and parenchymal inflammatory cells have not rigidly tried to categorize lung biopsy tissue as either DIP or UIP but emphasized gradations between the two and heterogeneity on the lung pathology. Generally, patients with midcourse disease have been studied.

A particular exception to this deemphasis of the predominant histologic pattern has been the work of Carrington and colleagues, reviewing principally the large patient population of Gaensler with chronic infiltrative lung disease. 130 cases were selected in which lung biopsy diagnosis and all the appropriate clinical, radiographic, and pulmonary physiologic studies were available for at least a one-year follow-up period or until death. Based on the criteria used to classify UIP and DIP well known to Carrington and to Gaensler, 93 cases were classified confidently as UIP (n = 53) and DIP (n = 40). Some 90 histologic features were said to be recorded. Among these carefully selected cases, some striking correlations were observed between patient outcome and tissue pathologic findings. Notably, some patients with UIP, if untreated, had spontaneous remission of disease, but most did not. Treatment with corticosteroids caused 62% of DIP patients to improve, but UIP was much worse in all respects. The experience offered in this study argues persuasively for a careful attempt at classifying patients based on open lung biopsy into the two histologic groups for prognostic and treatment purposes. The results also confirm Liebow and colleagues’ original postulate that DIP represented a distinct entity among the interstitial pneumonias. Balanced against this is the prevalent concept that DIP is just an early gradation within the evolution to UIP and does not separate patients with another form (and possible different etiologic agent) of disease.

In the interim, as forms of interstitial fibrosis have been recognized more frequently, it is evident that a spectrum of disease severity exists. More commonly, IPF is a chronic interstitial pneumonia, and patients have respiratory symptoms for months to several years before diagnosis, presenting themselves for medical evaluation at the midstage of disease. Survival afterward is in the range of 2–10 years. This more slowly evolving progressive disease in middle-aged adults is characteristic of most patients studied in recent clinical series.

Since very early, acute stages of IPF are observed rarely, usually a thorough clinical evaluation is made at established midstage disease. In staging it is important to determine the amount of inflammation and cellularity in lung tissue, especially the intra-alveolar or luminal portion. An active inflammatory stage generally reflects more recent disease and better prospects for a response to immunosuppressive therapy. Open lung biopsy tissue provides the most direct assessment of inflammation but is anatomically selective and is an invasive, once-only procedure. Consequently, indirect means have been developed to gauge inflammation in lung tissue, such as lavage of airways and alveoli and isotopic scans, which still must be evaluated for accuracy.

ASSESSING ALVEOLAR INFLAMMATION (ALVEOLITIS) WITH LAVAGE ANALYSIS

We initiated BAL studies in IPF patients to see if the recovered respiratory cells and alveolar lining fluid would give clues to the etiology and immunopathogenesis of the disease and to obtain macrophages for in vitro culture and biochemical study of their secretory products. Overall, the intent was repeatedly to sample the diseased airways and alveolar areas during the course of disease to monitor changes that might evolve. But an incidental, unexpected finding that the BAL cells contain an increased percentage of polymorphonuclear granulocytes (PMN) and eosinophils on differential count of the stained cell smear proved to be of more immediate interest. In contrast, BAL fluid cells from normal subjects, especially non-cigarette smokers, rarely contained a PMN (<1%) or eosinophil and smokers without overt bronchitis had few PMNs (5%–3% on differential count). Among the first 19 patients with IPF studied, seven were not receiving any therapy. They had a mean percentage of PMN among BAL cells of about 30%, ranging from 5% to 80%, which was independent of current smoking status. In those patients receiving corticosteroids (n = 13), the mean PMN was about 15, with a range of 3% to 50%. In both groups, the mean percentage of eosinophils was approximately 3% and was not altered by therapy. Alveolar macrophages, however, were the predominant cell in the differential counts, and their total recovery was twofold greater than usually obtained from normal persons. Lymphocyte percentages were variable but not distinctive, in contrast to the striking increase of these cells found in patients with sarcoidosis and chronic hypersensitivity pneumonitis. Enhanced amounts of IgG as a ratio with albumin were found in BAL fluid, but no other remarkable changes in other immunoglobulins or complement components were noted in IPF. These initial findings suggested the following avenues of
Turner-Warwick has addressed the current attitudes about PMNs, which perhaps has been an overplayed cell type in this situation, but also includes the combination of PMNs and eosinophils and a slightly enhanced count of basophil/mast cells. To see if this pattern of cell recovery pertained for another group of IPF patients, we summarized the differential cell counts for 70 IPF patients evaluated with lavage analysis in the past two years (Table 1). Patients are divided according to current cigarette smoking status, and no patient was receiving any anti-inflammatory or immunosuppressive therapy. Mean data are given, with the range of observations in parentheses. Cell counts have been grouped to dramatize several features of this kind of data presentation when individual cell percentages (and patients) are homogenized and reduced to mean values. The overall pattern of cells identified is still similar to the one noted originally in that the percentage of PMNs is high, even in nonsmokers, as are the percentages of eosinophils and basophils. Mean lymphocytes are increased somewhat in nonsmokers (up to 12% is within the normal range). As cell recoveries in general are 2- to 3-fold greater from IPF patients than from normal subjects, the absolute number of lymphocytes in BAL fluid increased, especially in the smokers. However, from the range of values found, it is apparent that any pattern of cells one may wish to find can be present. Some patients had a high percentage of lymphocytes recovered, yet could not be classified as having sarcoidosis or hypersensitivity pneumonitis, and others had many PMNs but no evidence of acute respiratory infection or airway irritation. The point is that within a large group of IPF patients, there are probably subgroups of patients. These may need to be separated on the basis of a special lung inflammatory response that reflects a different cellular reaction or indicates that the disease is at a different point in its evolution in that portion of lung sampled with lavage. Thus, by following a particular cell count in serial lavage analyses, varying correlations may result, and it is not certain which cell(s) best reflects the immunologic reaction for an individual patient. A comprehensive view of the cellular pattern or formula of the BAL cells is most important. When diffuse interstitial disease exists, we advocate lavaging at least two areas of the lung, perhaps in opposite sides, and processing the lavage fluid separately so that respective analyses can be made as an internal control to help corroborate results within each patient. Peripheral blood counts are needed also.

We have concentrated on the cells in lavage fluid and not delved into the enzymes and other proteins that can be measured in the acellular lavage supernatant. These components are equally important, and some of them are listed in

Table 1—IPF—Cell Count Differentials in BAL Fluid

<table>
<thead>
<tr>
<th>Percentages of Cells As</th>
<th>Macrophages</th>
<th>Lymphocytes</th>
<th>PMNs</th>
<th>Eosinophils</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non smokers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 23)</td>
<td>68 ± 4.2*</td>
<td>19 ± 3.2</td>
<td>10 ± 2.2</td>
<td>3.0 ± 0.9</td>
<td>0.9 ± 0.7</td>
</tr>
<tr>
<td>Smokers</td>
<td>76 ± 4.2</td>
<td>8.7 ± 1.8</td>
<td>11 ± 2.3</td>
<td>4.3 ± 1.2</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>(n = 47)</td>
<td>(23-90)</td>
<td>(6-66)</td>
<td>(4-43)</td>
<td>(0-15)</td>
<td>(0-5)</td>
</tr>
</tbody>
</table>

*Mean ± SEM and range observed.
Table 2—IPF—Cellular and Immunologic Changes

<table>
<thead>
<tr>
<th>Blood</th>
<th>Bronchoalveolar Fluid</th>
<th>Lung Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ig (IgG, )</td>
<td>Alveolitis (PMN, EOS but lymphs can be ↑)</td>
<td>interstitial inflammation</td>
</tr>
<tr>
<td>Immune complexes</td>
<td>Alveolar macrophages—Active and secretory components numerous;</td>
<td>Plasma cells</td>
</tr>
<tr>
<td>Cryoglobulins</td>
<td>Chemotaxins, IL-1</td>
<td>Fibroblasts</td>
</tr>
<tr>
<td>Serologic titers (low)</td>
<td>Plasminogen activator</td>
<td>Collagen synthesis</td>
</tr>
<tr>
<td>T-lymphs (sensitized type I collagen)</td>
<td>Fibronectin</td>
<td>(Type III&gt;1) fibrosis but no granuloma</td>
</tr>
<tr>
<td></td>
<td>Steroid-receptors ↑ ; mitosis ↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Collagenase; (PMN origin)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgG ↑, G.,</td>
<td></td>
</tr>
<tr>
<td>Immune complexes</td>
<td>IgG-releasing cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Histamine (mast cell mediators?)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2 and in the schematic Figure presenting current concepts of immunopathogenesis. In using lavage fluid analysis to monitor alveolar and perhaps interstitial events in IPF, counting cells only is too simplistic, and more appreciation of their dynamic interaction is needed. Quantitative assays for cellular mediators and secretory products that modulate the function of other cells are definitely promising and may prove to be better indices for judging disease or cellular activity. Recent publications about replication of macrophages, fibrogenic factors that affect fibroblast function, chemotaxins that attract inflammatory cells, circulating immune complexes, mediators such as γ interferon that stimulate cells, etc, or histamine levels

**LAVAGE CYTOLOGY AND TISSUE HISTOLOGY CORRELATIONS**

Whether the lung histology conforms to DIP or to UIP, judging the relative cellularity and inflammation in it seems important clinically. An open biopsy specimen provides a few grams of tissue from a specific lobe for this assessment, whereas lung lavage can sample the alveolar surface of a lobar subsegment and can be done in several anatomic locations, or a gallium citrate lung scan images the whole lung, but how well does intraluminal alveolitis mirror interstitial inflammation?

Davis and colleagues tried to correlate the airspace and interstitial mononuclear cells in lung biopsies and in lung lavage from a diverse group of patients with diffuse interstitial lung disease. The largest number had idiopathic, usual interstitial pneumonia (14 of 28), 7 specimens of normal lung were studied. The IPF biopsies contained more free airspace cells than normal tissue. As PMNs and eosinophils were rarely observed, lymphocytes were the cells emphasized in the morphologic analysis. In the airspaces about 10% of the free alveolar cells were lymphocytes in the IPF group (range, 1 to 22%), whereas the interstitial content based on a histologic estimate of inflammation varied from 1+ to 3+. The in situ correlation between alveolar and tissue lymphocytes did not reach statistical significance. Many of the patients underwent lung lavage simultaneously with open lung biopsy. A close linear correlation was found (p<0.001) between the percentages of lymphocytes seen in the airspaces and those recovered and counted in the lavage specimen. Thus, for these IPF patients, the intensity of the interstitial inflammation (based on lymphocytes) was not reflected well in the alveolar spaces, pointing to a discrepancy between cellular components of mural and luminal alveolitis; however, lung lavage did provide a representative sample of the free luminal cells. Several electron micrographs showed lymphocytes and macrophage migrating from interstitial to alveolar spaces, suggesting that cell movement into the airspaces occurred.

Haslam and colleagues undertook a 3-way analysis of inflammatory cells in lung tissue from 18 patients with cryptogenic fibrosing alveolitis who were found histologically to be in the UIP phase of disease. Some of these patients had associated collagen vascular diseases as included in the classification of fibrosing alveolitis that originated with Scadding and Hinson, who included patients with rheumatoid arthritis. Haslam and associates performed lung lavage on their patients two weeks before open biopsy was done. This study has been expanded now to include 20 patients. Lavage with about 300 ml of saline solution was performed in the lateral basal segment of the right lower lobe, the site of biopsy was reasonably similar to the area lavaged. Part of the biopsy specimen was formalin-fixed and prepared for histologic assessment; the remaining portion was minced and inflammatory cells extracted by agitating and pipeting the tissue fragments in buffered medium. Differential counts of cytacentrifuged stained cells from lavage and tissue extraction were compared with semiquantitative counts made in tissue sections. Lavage cell and biopsy extraction cell percentages correlated significantly for PMNs and eosinophils but not for lymphocytes. In CFA patients lavage underestimated the lymphocytes and plasma cells found in tissue. These investigators concluded that BAL cell findings reflected some of the tissue inflammation but did not give an exact representation of the total inflammatory components in the lungs.

Humminghake and associates also compared lung tissue and lavage inflammatory and immune effector cells in 9 patients with idiopathic pulmonary fibrosis. Both lung lavage (in B1 or B2 segment of left lung) and open lung biopsy were done in each patient, but the interval between the two procedures and whether the biopsy was taken from the area lavaged were not specified. The minced lung-teased tissue

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technique was said "to isolate almost all inflammatory and immune effector cells from the biopsies" according to the histologic analysis on residual tissue. In all the biopsies, these cells (PMNs, macrophages, and lymphocytes) composed more than 75% of the cells isolated. Cell extraction results were compared with lavage cells, but a formal tissue morphologic analysis, as in the Davis et al4 and Haslam et al5 studies, was not included. Moreover, individual values of cells in lavage and cells extracted from tissue were not compared for each patient. Given this limitation, the group data from IPF patients revealed almost perfect concordance for percentages of various cell types. Lung lavage (with 100 ml) retrieved about twice as many total cells as were extracted from a cm³ amount of biopsy tissue. Although tissue contained more lymphocytes than the lavages, the difference was not significant. For basophils, eosinophils, PMNs, and macrophages, lavage and tissue percentages were virtually the same. Likewise, T and B lymphocytes were in exactly the same proportions in lavage and biopsy. It was concluded that the alveolitis of IPF (and sarcoidosis) was characteristic of the inflammatory and immune effector cells present within lung parenchyma and that lung lavage accurately reflected the alveolitis.

**CELLS, PROTEINS, ENZYMES AND CELLULAR MEDIATORS RECOVERED FROM BAL FLUID THAT HAVE CONTRIBUTED TO IMMUNOPATHOGENIC CONCEPTS OF IPF**

Whereas analysis of cellular and protein components in BAL fluid may not give a specific diagnosis of IPF and its use in staging alveolitis and monitoring disease activity can be controversial, lavage provides cells suitable for *in vitro* culture from which a great variety of enzymes and mediators can be characterized. In acellular lavage fluid, representative of the alveolar lining secretions, immunoglobulins, proteases, phospholipids, and many other proteins also can be measured. These airside components plus some in the blood have been very helpful in formulating a sequence of disease pathogenesis despite the fact that the etiology of IPF remains elusive. Salient findings (see Table 2) from BAL fluid and of the alveolar macrophage put into culture can be increased. The significant increase in the recovery of alveolar macrophages correlates with the prevalence of these cells noted in histology of biopsies, particularly when more cellular forms of the disease are present. Appropriately, greater attention is being focused on the function of macrophages and a growing list of secretory products is being attributed to them. Several effector products and mediators produced by these phagocytes fit in very nicely to a scheme (see Fig 1) of pathogenesis that seems logical for this disease. Some caution is advised, however, for the activated alveolar macrophage put into culture can synthesize or release an enormous number of products. In the cellular supernatant fluid, or "the soup," one can assay what one wishes to find, it seems. Whether many of these macrophage products reflect unique derangements specific for certain diseases or are just part of an array resulting from nonspecific cellular activation needs more assessment. Many research studies have focused on a single or small selection of these macrophage products and, therefore, do not encompass a spectrum of assays that might address better this question of specificity. Of course, the availability of cells and supernatant specimens is a realistic limitation; however, miniaturized tests that require fewer cells and more specific bioassay and ELIZA methods suitable for measuring small quantities of mediators need to be developed. Then a more thorough analysis of mediators may be possible.

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**REFERENCES**

16. Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. J Lab Clin Med 1974; 84:559-73
17. Weinberger SE, Kelman JA, Elson NA, Young RC Jr. Reynolds
Concanavalin A Dependent Cell-mediated Cytotoxicity (CDCMC) in Bronchoalveolar Lavage (BAL) Fluid of Patients with Intersitial Lung Diseases (ILD)*

Evidence of Cytolytic T-Lymphocyte (CTL) Activity

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Previous investigations have demonstrated alterations in the composition and function of immune cells in BAL fluid of patients with certain ILD. However, the activity of CTL in these disorders has not been examined, although CTLs are one of the major effector cell components of immune responses. A problem in measuring the function of CTL in idiopathic ILD has been that the target antigen is unknown. To circumvent this difficulty, we used the CDCMC assay to measure CTL activity in patients with ILD. In this assay multivalent lectin binds to glycoproteins on both target cells and effector lymphocytes, causing their attachment in the absence of specific recognition of cell surface molecules.14

Materials and Methods

Peripheral blood lymphocytes (PBL) and BAL cells were obtained by standard techniques and enumerated by hemocytometry with a 0.1% crystal violet stain in 0.1 M of citric acid. Lymphocytes were purified by the nylon column technique. Cells were cultured in RPMI 1640 medium with 15% fetal calf serum, 2 mM of glutamine, and 100 µg/ml of streptomycin (RPMI complete). Polyclonal T-lymphocyte activation was induced by incubation with 20 µg/ml of concanavalin A (con A). One hundred microliters of viable lymphocytes were added to 100 µl of ⁶³Cr-labeled P815 cells, a murine mastocytoma cell line (for CDCMC) or K562 cells (for NK activity) in 96-well V-bottom microliter plates (Nunc). CanA, 20-40 µg/ml was included in CDCMC assays. Effector-to-target cell ratios were varied from 100:1 to 6.25:1, with the number of target cells kept constant. The effector cell concentration was 1.25 × 10⁵ cells/ml. After 3 hours at 37°C, 100 µl of supernatant from triplicate samples were harvested and the ⁶³Cr radioactivity determined in a γ spectrophotometer. Data are expressed as the percentage of cytotoxicity at effector to target ratios of 100:1.

Results

In preliminary studies with mitogen activated PBL, 20-40 µg/ml of con A was found to be the optimal concentration for CDCMC. To utilize the CDCMC assay to measure CTL activity in partially purified lymphocyte preparations, it must be shown that the lectin does not mediate NK activity. The NK cells were depleted from mitogen-activated cultures by treatment with the Leu P1 monoclonal antibody (Becton-Dickinson) and rabbit complement (C⁴). Antibody- and C⁴-treated cultures had about one third of the cytotoxicity on the

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