CHAPTER IV

DISCUSSION

Total and differential counts of the pleural fluid leukocytes could not differentiate tuberculous from malignant pleural effusions in our studies. Mononuclear leukocytes perdominated in both conditions and these cells were identified as T cells. Our results confirm other previous studies, showing that T cells are predominant in tuberculous and malignant pleural effusions (61-68). This probably reflects the ongoing cell-mediated immune response against the ivading intracelluar pathogen or tumor cells (59,60). In fact, substantial number of T cells in tuberculous pleural effusions were Ia+ (68), a marker of activated T cells (69). Antigen-stimulated T cell activation leads to lymphokihe production (24,26) and agegration of specific cytotoxic T Cells (43). The end results are the destruction or the elimination of pathogens or tumor cells as well as the untoward inflammatory reactions, such as granuloma formation (8,11).

Further phenotyping of the pleural fluid T cells revealed a higher percentage of helper T cells (OKT 4+) in the pleural fluids of both tuberculous and malignant patients when compared to peripheral blood (Figure 17). On the contrary, the percentage of suppressor/cytotoxic T cells (OKT 8 +) in the pleural fluids were significantly

results are in agreement with those of others that PPD-stimulated lymphocyte transformation (43,61,63,70,90) and LIF production (61,76) of PFMC are useful diagnostic tests for tuberculous effusion. PFMC from the group labeled as "non-specific" or "indeterminate" group behaved more like the tuberculous PFMC . This suggests that this indeterminate group is predominantly tuberculous as well and that our criteria for tuberculous effusion in this study were too stringent. Furthermore, our results indicate that in vitro PPD-stimulated lymphocyte transformation and LIF production of PFMC are of particular differential diasnostic value in borderlined cases of pleural effusion.

PPD-stimulated proliferation and LIF production of PBMC from tuberculous patients were much less pronounced than corresponding PBMC from tuberculous PFMC. These results are similar to those reported by Fujiwara et al (74). However, the responses were still significantly higher than those from nontuberculous PBMC. The value of the blood test alone in the diagnosis of tuberculous infection needs to be evaluated further. The tests are still quite cumbersome and ranges are quite narrow.

Besides LIF production, other lymphokines have also been shown to be specifically produced by tuberculous PFMC upon PPD stmulation. These include lymphocyte mitogenic

factor (74) and gamma interferon(67). If the assays for these lymphokihes can be made simpler in the future, such tests may turn out to be useful and practical diagnostic adjuncts in tuberculosis.

Like any other laboratory test, false - negative and false-positive tests have to be considered when interpreting results of PPD-induced functional tests of either pleural fluid or peripheral blood lymphocytes. Cell death is one of the common causes for a false-negative test. Technical failure (personal observation), specific unresponsiveness (anergy) to PPD, has been reported with tuberculous pleural fluid lymphocytes while their mitogenic response was intact (43). Such a specific unresponsive state has been found in advanced refractory or miliary tuberculosis. It may be due to antigenic overload (76) or due to suppressor T cells (77).

The explanation for the selective accumulation of antigen specific T lymphocytes in pleural fluid of tuberculous patients may be derived from our basic concepts of the immune responses. Antigen-specific T cells are activated by the incoming antigen via receptor (or receptors) for the antigen, and the major histcompatibility complex class II antigens present on the antigen presenting cells (78,84). The sensitized T cells then enter into the circulating pool and may be trapped or compartmentalized

at the site enriched with the antigen (79-80) or at any non-specifically induced inflammatory sites (81). It has been shown that peritoneal exudate cells (as induced by intraperitoneal injection of mineral oil, from guinea pigs sensitized with BCG by the subcutaneous route, are enriched with antigen-specific effector cells to the same extent as those present in the regional lymph nodes (81). In patients with tuberculosis, PPD-specific lymphocytes are concentrated in the regional lymph nodes (82). Similarly, PPD-reactive lymphocytes in the pleural fluid of patients with tuberculous pleurisy have been attributed to the same mechanism of preferential sequestration (83). In addition, the PPD-specific proliferative T cells in tuberculous effusion have been shown to be OKT4+ T cells since specific depletion of OKT4+ T cells, but not the OKT8+ cells, would abolish the PPD-stimulated proliferative response (68).

Figure 36 illustrates the hypothetical scheme of selective sequestratian of antigen-specific OKT4+ T cells from the circulating pool into the inflamed pleual space. The first few tuberculoprotein-specific T cells from the circulating pool enter the inflamed (infected) pleural space by leakage from the hyperemic blood or lymphatic vessels. Once these cells encounter the antigen in the pleura, many lymphokines are released such

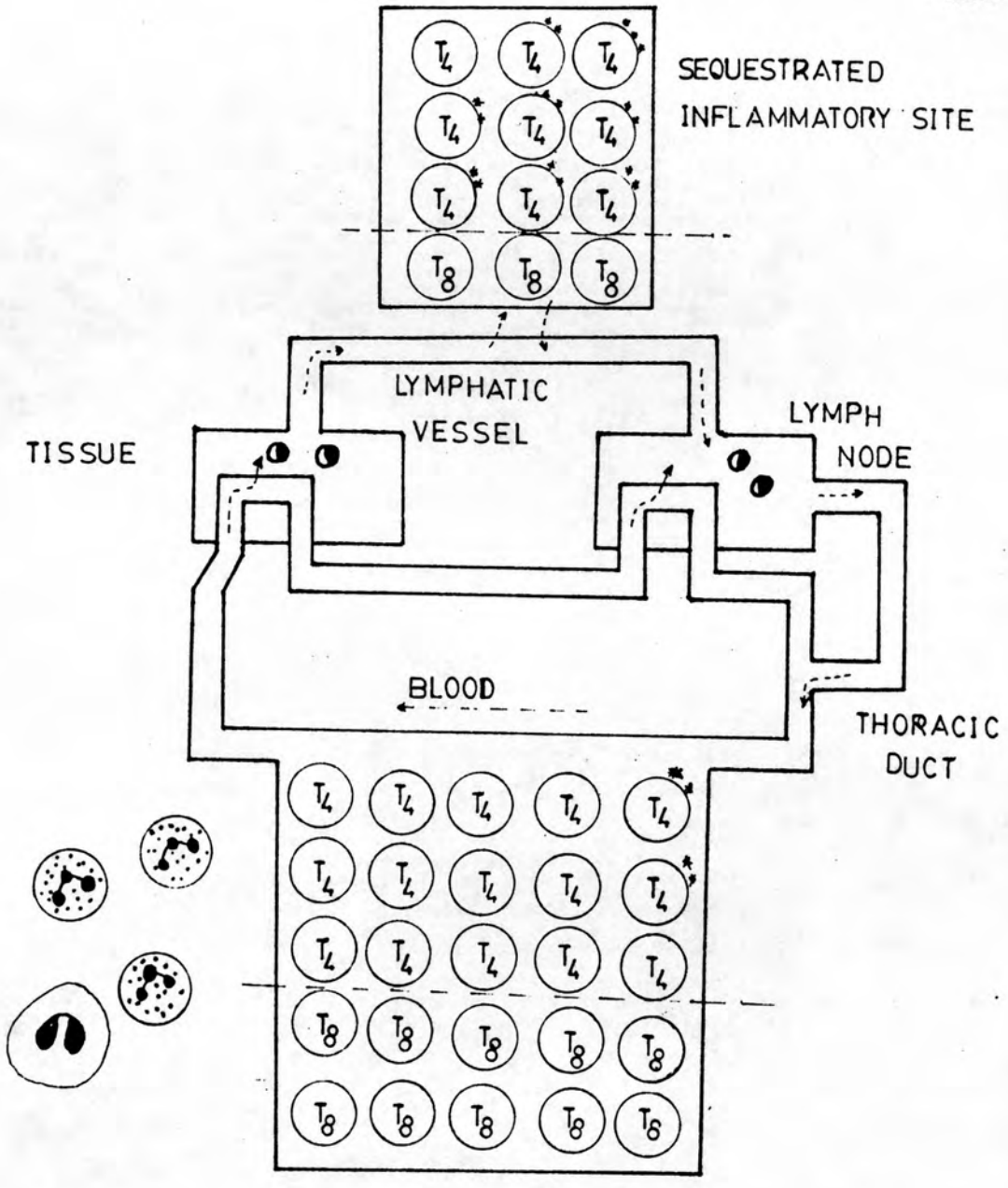

Figure 36. Recruitment of antigen specific T-cell subpopulations in inflammatory site from lymphocyte circulating pool.

as lymphocyte mitogenic factor, chemotactic factor, leukocyte migration inhibition factor and interleukin-2 (23). These lymphokines will then attract more antigen-specific effector cells (OKT4+ cells) as well as the non-specific inflammatory cells from the circulating pool into the inflamed pleural space. This will result in a higher proportion of antigen-specific OKT4+ cells in the pleural space. As a consequence, the proportion of OKT8+ cells in pleural fluid is decreased, resulting in a higher ratio of OKT4+/OKT8+ cells in pleural fluid.

Decreased response in PPD-induced functional assays of peripheral blood lymphocytes, as compared to the pleural fluid lymphocytes of tuberculous patients as observed in our study and in others (43,70,74), may be explained by the following :

(1) selective departure of tuberculoprotein-reactive effector T cells from the circulating pool into the inflamed pleural space.

(2) diluting out by other irrelevant mononuclear cells in the peripheral blood, whereas those in the pleural space are recruited by the specific antigen and therefore PPD-specific.

(3) presence of antigen-specific suppressor macrophages in the peripheral blood of tuberculous patients as reported by Ellner (43) and Fujiwara et al (74).

Attempts to demonstrate free LIF-like activity in pleural fluids, revealed that all of the specimens of tuberculous pleural fluid and 6 out of 9 malignant pleural fluid contained free LIF- like activity (Table 10). Thus, demonatration of free LIF-like activity in pleural fluid cannot differentiate tuberculous from malignant pleural effusions. This supports the findings of other investgators (72,75). The free LIF-like activity in our study consisted of both the heat-labile and heat-stable components (Figure 35). The heat -labile component may be the complement breakdown products as reported by Lew et al (85). This group of investigators found that tuberculous pleural fluids contained high levels of complement breakdown products of C3 and properdin factor B. These complement breakdown products were chemotactic for polymorphonuclear cells, i.e., posessing LIF-like activity. However, such chemotactants were readily destroyed by heating at 56 C for 30 minutes before LIF testing.

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On the other hand, the heat-stable component of LIF may be a true lymphokine. It reflects the in situ interactians between the tuberculous or carcinomatous antigens with the specifically sensitized lymphocytes present in the diseased pleural tissues. Spontaneous tritiated thymidine incorporatian, i.e., The endogenous turnover of tuberculous or malignant pleural fluid lymphocytes was higher than that of peripheral blood lymphocytes, indicating that the pleural fluid lymphocytes were already stimulated in vivo (72,86). Similary, many lymphokines have been defected in delayed type hypersensitivity reaction sites (87) as well as in joint fluids (88). Indeed, spontaneous release of lymphokines in malignant pleural effusian has heen considered by Petterson et al (75) to be a good prognostic sign. Failure to produce lymphokines in vivo correlated with a shorter survival of the cancer patients.

Conclusions

1. Phenotyping of pleural fluid mononuclear cells cannot differentiate tuberculous from carcinomatous effusions. T-cells and particularly the OKT4+ helper T-cells predominate in both forms of pleural effusion. They are antigenically-reactive in the case of tuberculous effusion.

2. In vitro PPD-stimulated lymphocyte transformation and LIF production using pleural fluid mononuclear cells are of added diagnostic value in differentiating some pleural effusions.

3. Assay of free LIF-like activity in pleural effusion is a simpler test than the direct LIF test. However, LIF-like activity, can be detected in all of the tuberculous effusion and in more than half of the carcinomatous effusions. Therefore, its role as a diagnostic tool is minor but it may reflect the prognosis of cancer patients and may have a role in immunotherapy.