Parallel tubular structures in lymphocytes

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Summary

This thesis is based on studies of lymphocytes containing parallel tubular structures (PTS) and of cell surface markers. Ultrastructurally PTS have been described as characteristic inclusions found in the cytoplasm of lymphocytes: bundle-shaped tubules orientated at random within one cell and even within one inclusion. The main purpose of the investigations was to answer the question to what population(s) of lymphocytes the PTS containing cells belong. In addition three chapters comprise studies on procedures used in connection with the submicroscopic examinations and in one chapter evidence for an exogenous influence on the occurrence of the PTS containing cell type is described.

In *chapter I* a general introduction is presented reviewing modern lymphocyte classification and its application to lymphoproliferative diseases.

In *chapter II* we report the distribution of different cell surface markers on peripheral blood lymphocytes in groups of healthy persons and patients with Hodgkin's disease, non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukaemia (CLL). The surface markers studied were receptors for sheep and mouse red blood cells (ESRBC and EMRBC), the receptor for the Fc fragment of IgG (Fcγ) as detected by the antibody-coated human erythrocyte rosette assay (EAHu) and surface membrane-bound immunoglobulin (sIg).
In CLL the proportions of cells with these markers differed significantly from those in healthy persons: small numbers of ESRBC rosette-forming cells (ESRBC-RFC) and EAHu-RFC, large numbers of EMRBC-RFC and sIg-bearing cells. In NHL only the percentage of sIg-bearing cells was increased in comparison with normal persons, while patients with Hodgkin's disease demonstrated a significant increase of EAHu-RFC.
Submicroscopic examination demonstrated a correlation between the occurrence of PTS and the presence of the Fcγ receptor: EAHu-RFC from healthy persons as well as from patients with Hodgkin's disease revealed the presence of PTS while non-rosetting lymphocytes lacked these structures.

In *chapter III* this relationship was studied in greater detail. In this study T, B and Null cell enriched subpopulations were obtained by means of ESRBC sedimentation and nylon wool adherence. The number of Fcγ receptor-bearing cells was determined by the antibody-coated human and ox erythrocyte rosette assays (EAHu and EAOx). The percentage of EA-RFC in the T cell fraction proved to be dependent on the procedure used to remove the rosetted sheep erythrocytes. Mechanical vibration resulted in considerably higher proportions of EA-RFC than osmotic shock or lysis with

ammonium chloride, probably due to a selective enrichment of T cells which form more labile rosettes.
Determination of the proportions of EA-RFC in the non-T, nylon wool adherent (B cell enriched) and the non-T, nylon wool non-adherent (Null cell enriched) fractions gave unequal numbers of EAHu-RFC and EAOx-RFC. In the B cell enriched fraction the percentage of EAOx-RFC was higher than that of EAHu-RFC (43% and 33%), in the Null cell enriched fraction the reverse was seen (45% and 54%).
Electron microscopic examinations of the EAHu-RFC and EAOx-RFC demonstrated that in all these fractions the majority of the EA-RFC contained PTS and/or associated amorphous electrondense granules. An exception were the EAOx-RFC in the B cell enriched fraction. In this cell population the proportion of EAOx-RFC displaying these structures (55%) was lower than in the case of EAHu-RFC (80%). This finding and the unequal percentages of EAHu-RFC and EAOx-RFC might reflect different avidities for different immune complexes, in this case EA's.

The above mentioned possibility for different avidities for different immune complexes together with contradictory data in the literature on the demonstration of Fcγ receptors on human peripheral blood lymphocytes prompted us to investigate different incubation conditions for both EA rosette assays. These investigations are described in *chapter IV*. It was shown that in healthy persons, in patients with Hodgkin's disease and in patients with NHL the mean percentages of EAHu-RFC increased significantly when the incubation was carried out overnight at 4°C instead of 1 hour at room temperature. This increase was caused by Fcγ receptor-bearing T cells. In the case of EAOx-RFC only a slight increase was found.
In addition differences were found between EAHu-RFC and EAOx-RFC in the various groups but also between patients and healthy subjects, e.g. the number of EAHu-RFC in patients with Hodgkin's disease was significantly increased in the overnight incubation in comparison with that of healthy persons.
In confirmation with the results in chapter II the mean percentage of EAHu-RFC was very low in the B cell CLL group, however that of EAOx-RFC was moderate to high. It was concluded that in the two rosette assays the antigen-antibody complexes may have different avidities to different lymphocyte subpopulations and that incubation conditions may influence this avidity.

In *chapter V* the ultrastructural features of T, B and Null cell enriched subpopulations are described. After removal of the sheep red blood cells from the ESRBC-RFC by lysis with ammonium chloride it was observed that the T cell fraction consisted mainly of two morphologically distinctive subsets. The majority of the cells displayed a smooth surface membrane and had a high nuclear to cytoplasmic ratio with few cytoplasmic organelles. A small proportion of cells was medium to large in size with a low nuclear to

cytoplasmic ratio and a relatively rough surface membrane. This cell type contained numerous cytoplasmic organelles including characteristic amorphous electrondense granules and sometimes PTS. The removal of sheep red blood cells by means of mechanical vibration resulted in a T cell fraction enriched in PTS and amorphous granules containing lymphocytes indicating that this cell type forms weak rosettes with ESRBC.

The non-T cell fractions were more heterogeneous in lymphocyte composition, including PTS containing cells. In the Null cell enriched fraction 50% of the cells contained PTS and amorphous electrondense granules, in the B cell enriched fraction 25%, though in the B cell fraction this cell type is probably due to contamination by Null cells.

It is suggested that the Fcγ receptor-bearing and PTS containing lymphocytes form a morphologically distinct subpopulation.

Submicroscopic studies on isolated T cell fractions gave some evidence for an influence of isolation procedures on the expression of PTS. We have therefore investigated the effect of the ammonium chloride buffer, which is applied to lyse sheep red blood cells. In *chapter VI* it is demonstrated that treatment with ammonium chloride buffer might be responsible for loss of the characteristic appearance of PTS.

In *chapter VII* a fixation procedure for immunofluorescence and immunoelectron microscopy is presented. It is demonstrated that fixation of tissues and cells with 1% paraformaldehyde does not seriously damage tissue and membrane-bound antigens. Addition of glutaraldehyde in very low concentrations (0.01-0.05%) improved the morphological conditions required for immunoelectron microscopy.

This fixation method was used to demonstrate T cell antigens by monoclonal antibodies (OKT3 and OKT8) on PTS containing lymphocytes from patients with chronic Tγ lymphocytosis, as is presented in *chapter VIII.*

Three patients with an expanded Tγ cell population revealed the same phenotype (OKT3+, 4—, 8+, I1—, M1—) and demonstrated a killer (K) cell function. Another patient with Tγ lymphocytosis showed a different phenotype (OKT3—, 4—, 8—, I1+, M1+) and demonstrated in addition natural killer (NK) activity. A patient with T-PLL had an OKT3+, 4+, 6—, 8—, 10— phenotype though a helper cell activity could not be demonstrated. Morphologically and cytochemically this case could also be distinguished from the patients with an expanded Tγ cell population.

The immunologic subdivision of the patients with Tγ lymphocytosis was not in agreement with the morphological and cytochemical results. Minor differences demonstrated by means of submicroscopic investigations did not parallel the immunologic subdivision. The only difference corresponding with the immunologic

characterization was the lack of parallel tubular structures in the lymphocytes of the patient with K/NK activity.

In *chapter IX* the effect is described of sera from patients with Hodgkin's disease on normal lymphocytes containing PTS. For this purpose normal donor mononuclear cells were cultured in the presence of patients' sera. The cultured cells were ultrastructurally compared with those stimulated with mitogens. The cells in mitogen stimulated cultures contained electrondense granules associated with multivesicular bodies. PTS could only be observed in lymphocytes which had not really changed their morphology.
About 50% of the sera from patients with Hodgkin's disease induced an increase in lymphocytes containing PTS and/or amorphous, electrondense granules (granular cells), paralleled by an increase in Fcγ receptor-bearing cells in the culture. This increase could not be correlated to the number of Fcγ receptor-positive lymphocytes in the patients peripheral blood.
A correlation was demonstrated between the increase in the percentage of granular cells and the patients clinical course, though an absolute distinction was not possible. If we take in account the presence or absence of splenic involvement we could not make an absolute distinction either.
Using positive control sera from different patients it is suggested that immune complexes, rheumatoid factor and anti-Epstein-Barr virus antibodies are not responsible for the observed increase caused by sera from patients with Hodgkin's disease.

In conclusion, the studies presented in this thesis have demonstrated that the PTS and amorphous electrondense granules containing lymphocytes form a morphologically distinct subpopulation which is present in the T an Null cell fraction. The expression of PTS may be influenced by isolation procedures e.g. treatment with ammonium chloride buffer. This morphologically distinctive cell type bears Fcγ receptors indentified by the EAHu-rosette assay, which appeared to be influenced by incubation conditions. Other assays for detecting Fcγ receptors may in addition identify high avidity Fcγ receptors on B cells.
The proportion of PTS containing lymphocytes is increased in the peripheral blood from patients with Hodgkin's disease and an expanded population of this cell type can be found in patients with Tγ lymphocytosis. In this last group of patients, PTS, the presence of Fcγ receptors and the presence of T cell antigens defined by the monoclonal OKT3 and OKT8 antibodies coincide. This finding was not demonstrated in the peripheral blood of healthy persons, suggesting the existence of different Tγ cell populations. This is supported by the phenotype of the patient with Tγ lymphocytosis demonstrating K/NK activity, whose cells also lacked PTS.

The investigations performed with sera from patients with Hodgkin's disease suggest the presence of serum factor(s) which may induce an increase in PTS and amorphous electrondense granules containing lymphocytes. Further studies are needed to identify the factor responsible for this phenomenon and to find out the origin and function of PTS.