Pha-Induced T-cell cytotoxicity. Mechanism and application in haemodialysis and renal transplant patients.
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SUMMARY

This thesis describes a method to measure PHA-induced cytotoxicity of human lymphocytes (nonspecific T-cell cytotoxicity), using $^3$H-thymidine prelabelled target cells (HeLa cells). The method has some advantages over the widely used $^{51}$Cr-release assay. Its application in two clinical conditions is also studied.

Chapter 1 describes the optimal conditions for the method. Labelling during 24 hours of 20,000 HeLa cells per well of a flat-bottom microtiter plate proved to be adequate. Stimulation with 10 µl PHA/ml of purified lymphocytes in three ratios of effector to target cells (2.5 : 1 - 5 : 1 - 10 : 1) during 24 hours gave the best measure of PHA-induced cytotoxicity. The reproducibility of the method was good. Supernatants of PHA-stimulated lymphocytes showed no cytotoxicity, indicating that lymphokines do not play a role. Mitomycin pretreatment had no effect, so the system is independent of DNA-synthesis. The presence of phagocytizing mononuclear cells in the lymphocyte suspension increased spontaneous cytotoxicity (without PHA stimulation) and reduced PHA-induced cytotoxicity, so that for adequate measurement of PHA-induced cytotoxicity these cells have to be removed.

Chapter 2 describes the effector cell, the influence of other cells and some functional aspects. The only cell effective in this test was the T cell. Granulocytes showed no cytotoxicity and did not reduce PHA-induced cytotoxicity as seen with monocytes. There was no influence of age and sex. Because the method is based on detachment of target cells, evidence is presented that real cytotoxicity is measured. Disruption of the nucleus was shown to occur. Protein synthesis is not needed, since the presence of emetine in the cultures did not reduce PHA-induced cytotoxicity. This supports once more the observation that
cytotoxicity in this system is not caused by lymphokines. It is very interesting that with emetine even a significantly higher cytotoxicity was found, suggesting that a suppressor cell which needs protein synthesis to exert its function is eliminated, although an effect on the target cell could not be excluded. Since the first description of suppressor cells by Gershon in 1971, several suppressor T cells against various functions have been described, such as suppression of antibody production, lymphocyte transformation and cytotoxicity. By using isolated T-cell suspensions we demonstrated the same increase in cytotoxicity, suggesting that the presumable suppressor cell in our system was also a T cell. In contrast to emetine, addition of vinblastin markedly reduced PHA-induced cytotoxicity. Vinblastin which is known as an antimitoticum also has a disrupting effect on microtubules. By this action, vinblastin can block the coordination of cell movement which is a function of microtubules and can thus inhibit the first step in the lytic event, explaining the reduced PHA-induced cytotoxicity.

Chapter 3 describes the results in haemodialysis patients with hepatitis B virus infection. We showed that there is a relationship between PHA-induced T-cell cytotoxicity and liver cell damage and recovery. This suggests that only haemodialysis patients who can mount a cytotoxic T-cell response can attack their infected liver cells. Patients who can not mount this response, do not have liver cell damage and remain carriers. The clinical outcome in haemodialysis patients is known to be different from that of acute hepatitis B patients who are otherwise healthy. Two possibilities could account for this difference. Firstly, although the same mean increase in percentage PHA-induced cytotoxicity was observed in haemodialysis patients and acute hepatitis B cases, there was a significant decrease in absolute lymphocyte and T-lymphocyte counts in haemodialysis patients. Secondly, serum inhibiting
factors could also make a contribution, as impaired T-lymphocyte proliferation during haemodialysis by inhibiting factors has been described. The basis of the carrier status in haemodialysis patients and normal persons seems to be different. In haemodialysis patients it more likely is a part of the general impairment in immune reactivity, whereas in normal persons it seems to be based on a specific defect against hepatitis B virus since they have a normal T-lymphocyte response against other antigens.

Chapter 4 describes the results in renal transplant patients. The clinical outcome of the 34 patients studied included two patients without rejection or infection, 21 patients with reversible rejection, 10 patients with irreversible rejection and one patient with cytomegalovirus (CMV) infection. Rejections occurred in the first two weeks, nephrectomy was performed in the second week on six patients, the other four were done in the third and fourth week. From the pretransplant values of PHA-induced cytotoxicity no differentiation could be made between these groups, but a clear difference is seen in the course of PHA-induced cytotoxicity. The patients without rejection or infection could be distinguished by a marked decrease of PHA-induced cytotoxicity (more than 20%) and the CMV infection by a marked increase (37%) in the first week. A clear difference in the course of PHA-induced cytotoxicity between the groups with reversible and irreversible rejection was seen in the second week (or 0-4 days before nephrectomy). A prospective study is worth to be done, in which two approaches may be followed: 1) To promote graft survival, the decision could be made to increase immunosuppressive therapy in patients with an increase of PHA-induced cytotoxicity above a certain level (e.g. 15%). 2) Prefering to decrease patients morbidity and mortality, the decision could be made to withdraw immunosuppressive therapy in patients with an increase of
more than 25% PHA-induced cytotoxicity, to remove the graft and to wait for another transplantation. CMV infection, the most common herpes virus infection after transplantation, may be recognized by a marked increase in PHA-induced cytotoxicity, but other viral infections may be differentiated only from rejection by addition of a specific T-cell cytotoxicity test to PHA-induced cytotoxicity.

This study has shown that this method to measure nonspecific T-cell cytotoxicity, clearly reflects the cytotoxic T-cell response in vivo in hepatitis B virus infection. It proves to be of value in the monitoring of immunosuppressive therapy in renal transplant patients, which is very important in addition to other parameters to improve the success of renal transplantation. It could be expected to be useful too in assessing the T-cell cytotoxic response in other pathological conditions.