SUMMARY

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Lymphocytes present within the tumor, commonly known as tumor infiltrating lymphocytes (TILs) are expected to be tumor specific immunocompetent cells. Being in close association with tumor cells *in vivo,* whether the TILs are sensitized against the tumor associated antigens (TAA), was investigated in the present study.

TILs were obtained from solid fibrosarcoma induced subcutenously by transfer of ascitic tumor cells in mice. For isolation of TILs from monolayer tumor cell suspension obtained after enzymatic digestion, both velocity sedimentation at unit gravity and centrifugation through discontinuous percoll gradient was considered. The isolation in percoll yielded better results than velocity sedimentation at unit gravity and was adopted in the present study (Fig. 2 and 8; Table 1 and 2). TILs collected from 55% interface mostly comprised of T cells (45 - 80%) and some NK cells (6 - 15%) (Fig. 9).

Kinetics of blastogenesis and DNA synthesis of TILs after activation with Con A *in vitro* were studied and these were compared to the results obtained with virgin lymphocytes and memory cells. The blastogenic response of TILs were at higher level than that of virgin lymphocytes, particularly at 12 and 24 hrs and its kinetics were similar to that of the memory cells (Fig. 10 to 13).

DNA synthesis was measured by incorporation of radioactive thymidine (^{3}H-TdR) in DNA. TILs and memory lymphocytes exhibit low profile of ^{3}H-TdR uptake in comparison to the virgin lymphocytes. Virgin lymphocytes incorporate maximum ^{3}H-TdR at 48 hrs which was

2 to 3 folds higher than that incorporated by TILs and memory lymphocytes at the same hour (Table 3 - 5). Similarity in kinetics of blastogenesis and low incorporation of ^{3}H-TdR by TILs and memory lymphocytes in comparison to virgin cells indicate that TILs may be sensitized cells like the memory lymphocytes.

It has been observed that virgin lymphocytes after activation require at least one cycle of DNA synthesis to become cytotoxic effector cells. Inhibition of DNA synthesis in them by 0.5 mM Hydroxyurea (HU) causes significant reduction of cytotoxic activity (Table. 9). On the contrary HU treated and untreated memory lymphocytes both express similar degree of cytotoxicity after restimulation (Table. 10). This indicates that DNA synthesis is not a prerequisite for memory cells to express cytotoxic function. TILs also showed similar results (Table. 11). It has been suggested that DNA synthesis possibly allows certain conformational changes in the chromatin during primary stimulation. Such changes seem to be stable and puts the memory cells and TILs at advantage in expressing cytotoxicity even without DNA synthesis.

BUdR at 50 μg/ml dose regime suppresses the expression of new genes in eukaryotic system, without affecting the genes which are already expressed. After activation, BUdR treated virgin lymphocytes show substantial reduction in cytotoxic activity in comparison to non treated virgin lymphocytes (Table. 12). BUdR treated memory lymphocytes did not show any significant reduction of cytotoxicity (Table. 13). Thus cytotoxic differentiation in virgin lymphocytes, not in memory cells, depend on new expression of certain genes. BUdR possibly does not affect the genes already expressed in memory cells.

Both BUdR treated and untreated TILs also express similar degree of cytotoxic activity (Table 14) indicating them to be in the category of sensitized cells.

Chromatin of memory lymphocytes is more susceptible to DNase I digestion than the chromatin from virgin cells (Fig. 15 to 17). DNase I is well known to cleave at the site of active genes preferentially. On DNase I treatment, memory lymphocyte nuclei released about 6% more DNA in the supernatant than that observed in case of virgin cell nuclei. This is likely due to the presence of more DNase I sensitive sites in the chromatin of memory lymphocytes, which in turn reflects the presence of more active transcriptional sites. This differential sensitivity of chromatin of virgin and memory cells bears considerable significance. It suggests the presence of more number of active genes in memory cells in addition to the house keeping genes that are essential to maintain regular routine activity. The differential sensitivity of chromatin from sensitized lymphocytes can be considered as a qualitative criterion to differentiate them from virgin lymphocytes.

Chromatin of TILs exhibit increased sensitivity to DNase I digestion like that of the memory cells (Fig. 18) This suggests that TILs harbour more active genes and in all probability are sensitized cells. The DNase I sensitivity of chromatin of tumor cells was also studied as because a few of them happen to be as contaminant in TIL population. In contrast to TILs, chromatin of tumor cells are less sensitive; even lower than that of the virgin lymphocytes.

From the above evidences it transpires that TILs are likely to be sensitized cells. Possibly that is why freshly isolated TILs expressed cytotoxicity against the tumor cells in ^{51}Cr-release assay (Table. 6).

Interestingly, TILs expressed higher degree of cytotoxicity after treatment with Con A *in vitro*.

In another set of experiments, activated TILs as such were adoptively transferred in mice bearing palpable tumors. It was observed the 37.5% of the mice undergoing treatment recovered completely (Fig. 19 and 20). In the remaining mice the rate of tumor development was slower during the initial weeks after treatment and their life span increased to an extent.