Recently, we reported the occurrence of the accelerated blood clearance (ABC) phenomenon of PEGylated liposomes (SL) upon repeated injection and there is a possibility of an interaction with an immune system to cause this phenomenon. In this study, therefore, we examined role of serum factor(s), spleen and effect of cyclosporin A (CsA) on occurrence of the phenomenon. Rats were treated with a first i.v. injection of SL (0.001 µmol lipid / kg body weight) and the second i.v. injection of [3H]-labeled-SL (5 µmol lipid / kg body weight) was administered at 5 days after first injection. In order to investigate involvement of serum factor(s) in the ABC phenomenon, we carried out transfusion experiments. The ABC phenomenon was evoked in normal rats by the transfusion of serum from rats received SL 5 days earlier, indicating that humoral serum factors are involved in causing the phenomenon. Subsequently we examined the role of spleen, since spleen is known for producing humoral serum factor(s) such as IgM, properdin, tuftsin. The ABC phenomenon was not observed in rat underwent splenectomy before the first injection. These results indicate that there is a possibility of involvement of serum factor(s) from spleen. In addition, we examined effect of CsA which is an immunosuppressant that mainly suppresses cellular immune system. The ABC phenomenon was not observed in rat treated with CsA. This study showed a possibility of involvements of serum factor(s) from spleen but not cellular immune system in occurrence of the ABC phenomenon.