Graft versus host reactions in the rat. An experimental study

Stet, Rene Josephus Maria

This study postulates that Graft versus Host Disease can, theoretically, be seen as the resultant of three vectors, which might be of crucial importance not only in the initiation but also in the manner of progression of the GvH reaction (GvHR). These vectors are firstly the T cell population and their phenotypically determined subsets (MRC OX-8^+ and W3/25^+ T cells). Secondly, the degree of MHC incompatibility (class I and class II) and lastly the target organ system involved. This latter vector comprises organs of both the lympho-hemopoietic and non-lympho-hemopoietic system. This thesis investigates the roles of these three vectors separately and/or in combination in a series of experiments designed to shed light on their relative importance in conditioning the nature of the GvHD.
Evidence is presented which indicates that it is the first two vectors that primarily determine the strength of the GvHR, whereas it is the third vector that determines the final outcome of a GvHD.

In chapter I a general introduction is given dealing with several aspects of GvHD such as the requirements for its induction and of the immunological mechanisms involved in its pathogenesis. In addition an overview of the MHC and T cell system in the rat is presented.

In chapter II the production and characterization of one of the monoclonal antibodies (Mab) used in this study is described. This Mab (U9F4) recognises a polymorphic determinant on certain class I MHC 45 kD molecules ($RT1^u$, $RT1^b$ and $RT1^c$). The nature of the binding of this Mab to various MHC haplotypes is investigated and it is established that the determinant is present on a RT1A encoded class I molecule.

In chapter III a description is given of a second Mab used in this thesis namely HIS 19. HIS 19 was selected from a panel of Mabs obtained after fusion of spleen cells of Balb/c mice immunized with Peyer's patch lymphocytes, enriched for germinal centre cells, with the myeloma X63. Data are presented which demonstrated that HIS 19 recognises a determinant present on a class II MHC molecule (33 kD). When HIS 19 was screened on a panel of different MHC haplotypes the data showed that HIS 19 recognised a class II polymorphic determinant. Class II molecules of the $RT1^n$ haplotype were, however, not recognised. It is further concluded in this chapter from

experiments using a MHC recombinant haplotype that the determinant recognised by HIS 19 is present on a RT1B encoded class II molecule.

In chapter IV the role of class I and class II major histocompatibility complex (MHC) antigens in T cell activation was studied by observing the effect of monoclonal antibodies (Mabs) which bind to these molecules. Two distinct activation pathways were used, namely mitogen stimulation using Concanavalin A (Con A), or alloantigen stimulation in mixed lymphocyte culture (MLC). The results showed that firstly in Con A stimulated cultures the presence of anti-class I or anti-class II Mabs, specific for the rat strain in culture, resulted in a dose-dependent inhibition of the proliferative response. Secondly, the presence of the anti-MHC Mabs during the MLCs, irrespective of whether they recognized determinants on stimulator, responder, or on both cell types, resulted in a dose-dependent inhibition of the proliferative response. Differences, however, in the extent to which they did so were observed. Possible mechanisms of this inhibition are discussed. It is suggested that that in the mitogen driven cultures anti-class I Mabs interfered with the IL-2 receptor expression, whereas the anti-class II Mabs most likely interfered with the response at the level of the accessory cells needed to provide the secondary signal IL-1. In the MLC those Mabs which recognized stimulator class I and class II molecules possibly prevented the recognition of the alloantigen and hence resulted in an inhibition of the response. Those Mabs recognizing only MHC molecules on the responder cells might have interfered with the proliferative response at the same levels as those described for the anti-class I and anti-class II Mabs inhibition of the mitogen driven cultures. In conclusion, it is suggested that these data indicate that MHC molecules might not only serve as restriction elements for $CD4^+$ and $CD8^+$ T cells but also may have a broader biological significance.

In chapter V the role of MHC class II expression was studied in different tissues prior to and during a GvHR. The GvHR across a full MHC barrier was induced by transfer of AO TDL into AOxBN F1 hybrid recipients. The hypothesis is erected that, similar to that observed in the in vitro equivalent of the GvHR, i.e. the mixed lymphocyte reaction (see chapter IV), class II molecules play an important role in the in vivo GvHR. Tissues were screened

during the GvHR using a panel of Mabs recognising either lymphocytes and their subsets or MHC class II molecules. The cellular changes in the tissues correlated with existing or de novo expression of class II molecules, epithelial cell types being those most severely affected. It is concluded that reactive T cells after being activated by class II^+ cells in the spleen recognise upon migration class II^+ cells (dendritic cells) in other organs. As a result of this local recognition soluble factors are produced which in turn cause de novo expression of class II molecules on epithelial cell types. This sequence of events leads to new targets for activated alloreactive T cells.

In Chapter VI the results of a screening for MHC antigen expression and the extent to which class I and class II expression changed in the liver during an ongoing GvHD is reported. The changes were separated into those related to the sinusoid-associated cells, including the liver parenchyma, and those related to the portal-tract-associated cells, including periportal hepatocytes. It is postulated that the observed increase in both number and class II expression of the Kupffer cells was most probably due to an increased phagocytic uptake of blood-borne cellular debris and was not the result of extensive damage to hepatocytes. In the portal tracts expanding infiltrates were found composed of class II^+ T cells and macrophages. These infiltrates are thought to probably have been due to a local accumulation of lymphocytes and macrophages as a result of an interaction of migrating donor-type alloreactive T cells with recipient type class II^+ cells present in the portal tract interstitium, which might have also interfered with normal recipient lymphocyte and macrophage traffic. Damage to portal-tract-associated cells was slight and confined to bile duct epithelial cells and to periportal hepatocytes. In conclusion, it is suggested that these changes do not indicate that damage to the liver parenchyma plays a major role in the pathogenesis of an acute GvH reaction.

In chapter VII the results of a comprehensive series of experiments designed to elucidate the importance of the three vectors referred to at the beginning of this summary are reported. To recapitulate these vectors are (1) the T cell population and their phenotypically determined subsets, (2) the degree of MHC incompatibility and (3) the target organ systems. In

the experimental design utilized, rats with differing MHC haplotypes were variously challenged by injection with either depleted or undepleted TDL. In addition the reponse of chimeric F1 hybrids of similar haplotypes allowed a separate investigation of the lymphoid and non-lymphoid target organ systems.

A synthesis of the resulting GvHD is presented in which the deterioration of the well being of the animals is alleged to have primarily resulted from their failure to take up sufficient food and water. The detailed study of the immunological mechanisms involved in the GvHR induced in injected rats supports, in large measure, the current notion of the MHC restriction of $CD4^+$ and $CD8^+$ T cells to class II and class I molecules respectively. However, the study also showed that $CD4^+$ cells can respond to class I alloantigens when in the presence of $CD8^+$ cells, the latter probably being responsible for the observed pathological disturbance. Also documented is the finding that $CD8^+$ T cell depleted TDL can, by themselves, react with class II alloantigens and result in a GvHD comparable to that seen with undepleted TDL. The underlying mechanisms of this process of disease progression are not, as yet, understood. It is suggested that they may be a suitable subject for further research.

Although MHC antigen differences are important in conditioning the nature of the resulting GvHD the results of the experiments using chimeras, where not only the initiation phase of the GvHD was compromised, but also the range of target organs restricted to the non-lympho-hemopoietic tissues, showed that in all instances histological signs of a GvHR were observed. These findings suggest that the absence of incompatibility in the lympho-hemopoietic tissues does not abrogate the onset of the reaction. Even in the absence of both LHPS derived and intrinsic MHC alloantigenic stimulation in the non-LHPS ([AO bone marrow -> AOxRP] chimeras) a GvHR was observed. The nature of the process intiating this reaction is unknown.

In chapter VIII the binding in the rat thymus of injected monoclonal antibodies (Mabs) was studied. The Mabs, injected either intravenously or intraperitoneally, penetrated the thymus initially across the thymic capsule from the extravascular space. The extent to which the Mabs penetrated both cortex and medulla correlated with serum presence of the Mab. These results suggest that the thymus cortex and medulla are permeable to Mabs

present in the extravascular compartment. Were this situation a general phenomenon, which applied to circulating self non-MHC antigens under physiological conditions, the current dogma relating to induction and maintenance of self-tolerance would need to be reviewed.

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