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The second signal (costimulation):

Binding of maleyl-BSA to different cellular populations:
This study is in two parts, addressing issues in antigen presentation and T cell activation. One part attempts to investigate the basis of non classical activation of a T cell hybridoma. The second part examines the basis of quantitative and qualitative changes in the immune response with respect to the presentation of MHC-peptide complexes to exogenous antigens.

**Nonclassical ligand activating a T cell hybridoma:**

The activation of a T cell hybridoma, 1E3, has been explored. It was generated from H-2\(^k\) mice immunized with mouse L cell fibroblasts transfected with I-A\(^b\). The reactivity of 1E3 to L cells is dependent on MHC class II molecule, but is not restricted by classical MHC molecules. It is known from many studies that nonclassical MHC molecules play very important role in the host defence mechanism and such molecule may be involved in activating 1E3. The requirements for the activation of 1E3 were therefore investigated.

1E3 recognizes I-A\(^b\), I-A\(^k\) and I-E\(^b\) transfected L cells, but not parental L cells. However, I-A\(^k\) is a self allele for mice from which the 1E3 was generated. In addition, I-E\(^b\) is a different isotype of MHC class II from the I-A molecule present on the L cells which were used for immunization. These previous data suggested that allelic restriction for the activation of 1E3 appeared to be degenerate, but it was MHC class II dependent. 1E3 was also shown to be activated by LPS-treated spleen cells from the H-2\(^d,k\) and \(i3\) haplotypes of mice [Kumar 1994].

**1E3 recognizes LPS-treated spleen cells or peritoneal exudate cells:**

Normal spleen cells do not express the stimulatory ligand for 1E3; however, they do so after LPS activation. It was interesting to investigate
other conditions in which spleen cells expressed the stimulatory ligand for 1E3. Spleen cells treated with IL-4, IFN-γ or PMA did not stimulate 1E3 (figures 1 and 2). The specificity of the cell type stimulating 1E3 in the LPS-treated spleen cells was analyzed. It was observed that, while LPS-treated WEHI 274.1 cells (macrophage cell line, H-2^d^) stimulated 1E3 (figure 3A), a B cell line, A20, did not even after LPS, IFN-γ, IL-4 or PMA treatment (figure 3). Subsequently it was found that LPS-treated-PECs also activated 1E3 (figure 4). Further characterization of the ligand for this hybridoma would have been facilitated by a mouse strain which does not express the stimulatory ligand for 1E3 on its splenocytes even after LPS-activation. LPS-treated spleen cells or peritoneal exudate cells from *M. mus. musculus* mice of MHC haplotypes H-2^b^, q, r, s, v all stimulated 1E3 (figures 7 and 8). In addition, LPS treated PECs from *M. mus. domesticus* (AKR/J) mice also stimulated 1E3 (figure 9). The stimulatory molecule for 1E3 was cell surface associated, not secreted, as both paraformaldehyde fixed LPS-treated macrophages and L-Ab cells stimulated 1E3 (figures 6 and 16).

1E3 reactivity is not restricted by MHC class II or by super-antigen:

The degeneracy of allelic restriction for MHC class II implies that 1E3 recognizes a super-antigen like molecule, expressed by L cells and LPS-treated macrophages. In addition LPS-treated PECs from various mouse strains (figures 7, 8, 10, 11) also activate 1E3. These data argue a possible involvement of a super-antigen like molecule in 1E3 activation. Super-antigens are known to be presented in the context of MHC class II and the responses induced by them can be blocked by anti-MHC class II antibodies. Data observed here suggest that MHC class II based superantigens are not involved in the activation of 1E3, since two different monoclonal I-A^b^ reactive antibodies failed to block the response of 1E3 towards L-A^b^ cells.
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(Kumar, 1994), and since LPS activated PECs from the I-Aα deficient mice (which lack any cell surface MHC class II expression) also stimulate (figure 12). In addition these data also confirm that cell surface expression of the MHC class II molecule is not required for 1E3 activation.

1E3 response is not restricted by MHC class I either:

Since the activation of 1E3 is dependent on MHC class II and not restricted by it, it is possible that 1E3 recognizes a conserved peptide epitope from the MHC class II molecule in the context of MHC class I. The fact that LPS-treated PECs from mouse strains of various MHC haplotype activate 1E3 rules out the possibility that response of 1E3 is restricted by the highly polymorphic classical MHC class I molecules. Earlier work from this laboratory has shown that an anti-H-2KkDk specific monoclonal antibody Y-3 failed to block the response of 1E3 (Kumar, 1994). This was further supported by the observation here that LPS treated spleen cells or PECs from β2-M or TAP-1 deficient mice also stimulated 1E3 (figure 10 and 11), although these PECs are defective in presentation of the many MHC class I binding epitopes [Bender et al., 1992; Attaya et al., 1992; Powis et al., 1991; Spies et al., 1992; Kelley et al., 1992].

1E3 recognizes a highly conserved ligand:

It is possible that the restriction element for the 1E3 is a nonclassical MHC molecule, especially because they are less polymorphic and have less dependency on β2-M [Balk et al., 1994] than class I class molecule do. In addition, some of them do not depend upon a functional TAP complex for peptide loading and cell surface expression. However, LPS-treated human plastic adherent peripheral blood mononuclear cells also stimulated 1E3
(figure 17). Although an MHC molecule which is conserved (at least in functional terms) in human and mouse is not known, its existence cannot be ruled out altogether. It may also be that 1E3 activation does not occur through MHC-like molecules, but through some other molecule involved in cell-cell interaction (such as costimulatory molecules). However, no costimulatory molecule alone has been shown to be capable of transducing signals to T cells to produce factor/s such as IL-2 which can support CTLL-2 proliferation so far.

I-Aα deficient macrophages stimulate 1E3 on being LPS activated, but only MHC class II expressing L cells can stimulate it. Based on this, it can be argued that the 1E3 stimulatory ligand is different between L cells and macrophages, or that cytosolic I-Aβ or I-Eβ expressed in I-Aα deficient PECs is enough for the expression of the 1E3 stimulatory ligand. Since rat-anti-L cell antiserum blocks the response of 1E3 to L-Ab cells, parental L cells probably express some molecule which is involved in the activation of the 1E3, though it is not yet clear how MHC class II molecules in L cells help this molecule to become a stimulatory for 1E3.

**1E3 has unusual activation receptor:**

When 1E3 was analyzed for its T cell receptor (TCR) expression by flow cytometry and functional assays, it was found that the expression of TCR-CD3 complex (figure 16) was undetectable on the 1E3. Anti-CD3ε and anti-TCRβ antibodies failed to stimulate 1E3, even in the presence of accessory cells such as the macrophage cell line WEHI 274.1 (figure 17). This observation is unexpected, but the expression of Thy-1 antigen still shows its T cell nature (figure 16). However, 1E3 is different from its parent BW5147 in that LPS-treated PECs do not stimulate BW5147 (figure 5).
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Regulation of T cell response by antigen presentation:

In the second study, the changes in the immunogenicity by increasing the number of MHC-peptide ligand complexes on the APCs in vivo has been studied by using targeted delivery of antigens to scavenger receptors. Exogenously administered soluble antigens have a limited ability to generate an immune response in vivo; a problem usually overcome by the use of adjuvants [Claassen et al., 1992]. In the present study antigens were chemically modified (maleylated) to make them scavenger receptor ligands and the immune response generated after immunization with native and maleylated antigens in the absence of adjuvants has been analyzed.

Maleylated proteins are better immunogens than their native counterparts:

The data presented here show that immunization with maleyl-DT in PBS elicits a better proliferative response than DT-PBS (figure 18), indicating the enhanced immunogenicity of maleyl-DT in vivo. This supports the earlier finding in the laboratory that maleyl-proteins generate a better antibody response to themselves than their native counterparts do in vivo. The proliferative response of spleen cells from maleyl-DT immunized is much better than that of DT-immune mice to DT (figure 21). This suggests that maleyl-DT generates a better immune response for DT than DT does for itself.

The relative contribution of T cells and B cells in the enhanced proliferation induced in maleyl-DT immune spleen cells by maleyl-DT was also analyzed. The haptenic maleyl group on the maleylated antigens makes them polyvalent B cell antigens, so that it is possible that enhanced proliferative responses seen in maleyl-DT immune cells to maleyl-DT is due at least in part to B cells. Maleyl-DT and maleyl-TT share maleyl groups as
polyvalent haptens for B cells (Abraham et al., 1995). However, maleyl-TT cannot induce proliferation of maleyl-DT immune spleen cells (figure 23A), though it does so for maleyl-TT immune spleen cells (figure 23B). This observation suggests that B cell activation is not responsible for the enhanced proliferation seen in the maleyl-DT immune group, although T cell dependent activation of B cells can not be ruled out in the above case and it may contribute to some extent in the proliferation seen above.

The antibodies generated after immunization with maleylated antigen do not cross-react significantly with native antigen, but do so with other maleylated antigens. In contrast, DT induces a significant T cell proliferative response in maleyl-DT immune spleen cells and vice-versa (figure 21). This is true for other antigen pairs such as OA and maleyl-OA, CA and maleyl-CA, BSA and maleyl-BSA, where the stimulation of monoclonal T cell lines were used as a read-out (figure 20).

The primary sequence of DT contains 38 lysines, which are the target of maleylation. Some lysine residues will be already modified in DT (because of formaldehyde treatment, during which diphtheria toxin is converted to diphtheria toxoid), but many lysine residues are free as seen by a positive trinitrobenzene sulfonic acid (TNBS) reaction, and are free for maleylation. However, despite this, polyclonal T cell responses are cross-reactive between DT and maleyl-DT. Therefore, either T cell epitopes in DT do not have lysine residues or such lysine residues do not make any significant difference towards recognition of MHC-peptide by TCR, or a residual proportion of DT in maleyl-DT is not maleylated, or a given lysine residue is not modified in each and every DT molecule by maleylation, and this is responsible for the cross-reactivity.

If any of these are correct, the T cell repertoire in the maleyl-DT immune spleen cells should be smaller for DT as compared to maleyl-DT. When receptor-mediated endocytosis of maleyl-DT was blocked in the
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proliferation of maleyl-DT immune spleen cells, the proliferative responses to DT and maleyl-DT were comparable (figure 24). These data suggest that most if not all of the T cells primed in vivo by maleyl-DT can recognize DT. This implies that cross-reactivity between DT and maleyl-DT at the T cell level is not primarily because of any partial maleylation of DT or related possibilities.

Another possibility is that the maleylated proteins are demaleylated during processing. The most likely compartment in the cell where demaleylation can occur is the endosomal/lysosomal compartment, since maleylated proteins are reported to be demaleylated at acidic pH [Haberland et al., 1985]. The BSA specific T cell hybridoma used here recognizes the BSA peptide 141-153 (4) on I-E\(^b\) and this peptide has one lysine. The BSA specific hybridoma can be activated by maleyl-BSA better than by BSA (figure 20D). Similar results were also obtained with the CA specific T cell line, D10.G4.1 and two OA specific T cell hybridomas, 13.8 and 23.3 (figure 20A, B and C). However, the epitope of D10.G4.1 in CA does not have any lysine residue and the peptide epitopes of 13.8 and 23.3 are not known. These data together also suggest that maleylation probably does not interfere with the processing of the proteins in the endosomal/lysosomal compartment.

Native and maleylated proteins cross-react with each other at the T cell epitope level in vivo. Both maleyl-DT and DT can recall the proliferative response of the polyclonal T cells primed with DT 201-222 (figure 22A). Similarly, a universal TT epitope, TT 830-844, induces a proliferative response in maleyl-TT immune spleen cells significantly but not in TT-immune spleen cells (figure 22B), showing that maleyl-TT primes for this non maleylated T cell epitope better than TT does.
Enhanced presentation of maleylated proteins to T cells over their native counterparts:

An important feature of the result here is the better stimulation of T cells by maleyl-antigens. This was true for the all antigen pairs (e.g. OA/maleyl-OA, BSA/maleyl-BSA, CA/maleyl-CA, DT/maleyl-DT) and with all types of T cells, such as polyclonal \textit{ex vivo} T cells, monoclonal T cell clones or hybridomas (figures 20 and 21). It is possible that better uptake of the maleylated proteins occurs through receptor-mediated endocytosis, while the native proteins are taken up by pinocytosis which is a relatively inefficient pathway for uptake. This may be responsible for the enhanced T cell response induced by maleylated proteins as compared to their native counterparts. This is borne out by the finding that enhanced proliferation of maleyl-DT immune spleen cells to maleyl-DT over DT can be significantly blocked by the addition of maleyl-BSA (a scavenger receptor ligand) in a dose dependent manner (figures 24A and 24B). The addition of maleyl-BSA does not affect DT induced proliferation (figures 24A and 24B). Similar results were obtained when the presentation of maleyl-CA and CA to D10.G4.1 was studied (figure 25). These data suggest that uptake of maleyl-proteins by scavenger receptor mediated endocytosis leads to their better presentation by APCs, over native antigens.

Qualitative effects of maleylation of immunogens on the immune response:

T helper cell responses are of two kinds, type-1 and type-2, based on the nature of the cytokines secreted by them [Kim \textit{et al.}, 1985, Cherwinski \textit{et al.}, 1987]. Type-1 T cell responses are dominated by the production of IFN-\(\gamma\) and TNF-\(\beta\), while IL-4, IL-5 and IL-10 dominance constitute the type-2 T cell response. There was a distinct possibility, that targeting maleyl-antigens to
the scavenger receptor bearing APCs (presumably macrophages) may change the cytokine profile of the primed T cells. When the culture supernatants of DT or maleyl-DT-immune spleen cells cultured with antigen in vitro were analyzed, a relative predominance of IFN-γ over IL-4 and IL-10 in the maleyl-DT-immune group as compared to the DT-immune group was observed (figures 28 and 29). The results observed for the antigen pair CA and maleyl-CA followed a similar pattern (figures 30 and 31). The relative cytokine profiles remain unaffected by use of the either native or maleylated antigens for in vitro recall experiments. These data are consistent with the finding that antibodies generated after immunization with maleylated proteins show a predominance of IFN-γ dependent isotype IgG2a over IL-4 dependent isotype IgG1, in comparison with the antibodies generated after immunization with native antigen [Abraham, 1996].

Possible reasons for the change in the immune response by maleylated immunogens:

What are the observed differences (qualitative and quantitative) in the immune response elicited by the native and maleylated antigens? Both the first signal (MHC-peptide complex) as well as the second signal/s (costimulatory signal/s) delivered to the T cells can affect the outcome of the T cell-APC interaction [Liu et al., 1992; Steinman et al., 1991].

The first signal (MHC-peptide complex):

The encounter of protein antigens with APCs results in internalization of the antigens, and subsequent degradation into smaller peptide fragments which are loaded on to MHC molecules and are presented to T cells. The MHC-peptide complexes on the surface of the APCs are recognized by T cells
through their clonotypic antigen receptor, the TCR. After interaction with the MHC-peptide complex, TCRs transduce the signal/s leading to T cell activation.

There are reports suggesting that the number of T cell receptors engaged by the MHC-peptide complexes affect the T cell response. The number of appropriate MHC-peptide complexes also influences the positive or negative selection of T cells in thymic organ culture [Sebzada et al., 1994]. Viola et al. (1996) have shown that a threshold number of TCRs have to be downregulated after interaction with MHC-peptide complexes for successful T cell activation. Therefore, the relative numbers of relevant MHC-peptide complexes on spleen cells from the mice injected with the CA or maleyl-CA were examined over a period of the time.

The MHC-peptide complexes were detected by the level of stimulation of the monoclonal T cell line D10.G4.1, which is specific for CA. Figures 34 and 35 show that at all the time points observed (between 3 hours to 72 hours post injection), higher levels of stimulatory ligands (MHC-peptide) were present in the maleyl-CA group than in the CA group. No stimulation was observed after 96 hours in either group. The observation here that these MHC-peptide complexes have short half-lives is consistent with some reports [Adorini et al., 1989; Harding et al., 1989; Mueller et al., 1993] but not with the others [Lanzavecchia et al., 1992; Nelson et al., 1994].

These data support the hypothesis that a higher ligand (MHC-peptide) density leads to the generation of a relatively type-1 T cell response [Pfeiffer et al., 1995]. However, Parish and Liew (1972) have shown that immunization with low or high doses of modified Salmonella flagellin antigen elicits a better delayed type hyper sensitivity reaction (an indication of type-1 T cell response), while an intermediate dose favors better antibody production (an indication of type-2 response, though isotypes were not analyzed). Normally BALB/c mice mount a type-2 T cell response to L.
major and are susceptible to the pathogen. Bretscher et al., (1992) have also shown that immunization with a low dose of Leishmania major leads to a type-1 T cell response in BALB/c mice, making the immune mice resistant to challenge infection. However, these studies involve the use of pathogens such as L. major, parts of pathogens such as Salmonella flagellin or adjuvants like CFA, all of which are capable of activating the immune system in different ways, which may affect the outcome of an ongoing immune response. This may explain some of the discrepancies observed in these studies. However, the present study does not involve the use of pathogens or antigens derived from pathogens and attempts to detect any possible non-specific effects of scavenger receptor ligation on the immune response did not show any significant changes.

The second signal (costimulation):

The ligation of the scavenger receptors on macrophages leads to the secretion of prostaglandin-E2 (PGE2) and IL-1 [Hamilton et al., 1987; Palkama, 1991]. Both these molecules have been shown to modify T cell responses [Minakuchi et al., 1990; Koide et al., 1987]. Thus, it is possible that the ligation of the scavenger receptor changes the costimulatory capacity of the APCs. These changes may be associated with altered levels of IL-1, PGE2, CD40, CD80, CD86 or CD54, all of which can be assayed directly. But even after such changes the question will remain, do these changes modify T cell priming? It is also possible that some other changes occur which affect the priming. It was essential to analyze whether changes in the costimulatory capacity of the APCs by scavenger receptor ligation could modify the T cell response. Therefore, the effect of scavenger receptor ligation on the T cell response was analyzed in a functional assay.
The stimulation of the T cell clone D10.G4.1 by CA presented by PECs (figure 25), or spleen cells (figures 42 and 43) was analyzed in the presence or absence of maleyl-BSA. It was observed that presence of maleyl-BSA caused no significant differences in the proliferative response of D10.G4.1. Enhanced presentation of maleyl-CA over CA was blocked by the addition of maleyl-BSA in the same assay, showing that scavenger receptor bearing APCs were present and maleyl-BSA was binding to the scavenger receptors (figure 25). The data obtained when DT or maleyl-DT was used to stimulate maleyl-DT-immune spleen cells in the presence or the absence of maleyl-BSA (figure 24) showed a similar pattern.

The costimulatory requirements for naive and memory cells are different [Luqman et al., 1992; Velde et al., 1993; Croft et al., 1994]. It is possible that scavenger receptor ligation on APCs may modify the response of naive T cells, but not of memory T cells. This possibility was analyzed by stimulation of thymocytes (a source of naive T cells) with anti-CD3ε antibody, when the APCs were plastic adherent peritoneal resident cells, in the presence or the absence of maleyl-BSA. The presence of maleyl-BSA (where the APCs were untreated or pretreated with maleyl-BSA) does not cause a significant difference in anti-CD3ε induced proliferation of syngenic thymocytes (figure 26). The effect of scavenger receptor ligation during in vivo priming was also analyzed by immunizing mice with DT alone or coimmunizing mice with DT and maleyl-TT. The spleen cells from these immune mice were cultured with DT in vitro and the proliferation of splenocytes was studied. Figure 27 shows that DT induces comparable proliferation in both DT or DT plus maleyl-TT immune spleen cells. These data suggest that the ligation of the scavenger receptor does not affect T cell priming with respect to proliferative responses.

Discussion
Binding of maleyl-BSA to different cellular populations:

Scavenger receptors (SRs) were originally defined by their ability to bind acetylated- or oxidized low density lipoproteins (LDL). Apart from these ligands, SRs also bind to a variety of polyanionic ligands such as poly-G, poly-I, maleyl-BSA and other maleylated proteins. Until recently, it was known that maleyl-BSA binds to macrophages and to certain endothelial cells, but in the last two years many other cells such as certain dendritic cells, B cells, platelets and adipocytes have been found to express cell surface molecules (such as CD36, CD32, CD68), which exhibit binding properties similar to scavenger receptors. Therefore, the study of the binding of maleylated proteins to cells of different lineages was worthwhile.

Maleyl-BSA binding to cells was detected by a rat anti-maleyl-protein antiserum. This anti-serum shows equivalent binding of the maleyl-BSA to parental CHO cells or CHO cells transfected with the SR-AI or SR-AII molecules (figure 36A, B and C). It was essential to analyze if this antiserum binds to maleyl-BSA bound on the cell surface or to the other cell surface associated molecules. A positive signal was detected only when maleyl-BSA (50-100 μg/ml) was used in the staining, and no significant difference in the signal was observed between unstained cells and cells incubated with the BSA, the anti-serum and the detecting fluorochrome conjugate (figure 36D, E and F). The binding of maleyl-BSA to the transfectants can be inhibited by pretreatment with poly-G (figure 36B and C), whereas the binding of maleyl-BSA to parent CHO cells is unaffected by poly-G (figure 36A), a ligand for SR-AI/II. Ex vivo T cells did not show any binding to maleyl-BSA using the same detection system (figure 39F). These data show that this anti-serum can detect the binding of maleyl-BSA to the cells, though the nature of cell surface molecule/s (other than SR-AI/AII) to which maleyl-BSA binds remain unclear. This antiserum also detects the binding of maleyl-BSA to
plastic adherent peritoneal exudate cells as well as to the macrophage cell line WEHI 274.1 (figure 37).

Dendritic cell enriched spleen cells and total spleen cells were analyzed for their ability to bind to maleyl-BSA in two color flow cytometry. A fraction of maleyl-BSA positive cells were comprised of dendritic cells (N418 positive cells) (figure 38A). Similarly, around 20-30% of the dendritic cells (N418 positive cells) stained for maleyl-BSA (figure 38B) in the dendritic cell enriched spleen cell preparation. However, almost all B cells (IgM+ cells) in the spleen were found to be positive for maleyl-BSA binding (figure 39E), whereas T cells do not bind to maleyl-BSA (figure 39F). It was imperative to determine whether such binding is accompanied by internalization and moreover whether it contributes to the generation of MHC-peptide complexes. These possibilities were analyzed by using dendritic cell- and macrophage-depleted spleen cell or a B cell line, CH-1, to present CA and maleyl-CA to D10.G4.1. It was observed that both the APC populations presented maleyl-CA better than CA, and this effect was blocked by the addition of maleyl-BSA (figure 42 and 43). Other experiments done in the laboratory using splenic dendritic cells as APCs have also yielded similar results. These data show that maleyl-BSA, maleyl-CA and consequently other maleylated proteins also bind to B cells, to a fraction of dendritic cells and are subsequently internalized.

Certain implications of these data can be summarized. It has been shown that dendritic cell mediated priming is essential for the priming of T cells [Inaba et al., 1990; Levin et al., 1993]. It is known that follicular dendritic cells in germinal centers of human lymph nodes can be stained with anti-SR antibody [Geng et al., 1995]. Recently, it has been shown that CD4+ CD11C+ CD3- dendritic cells in the germinal centers express MHC class II molecules and are strong stimulators of T cells [Grouard et al., 1996]. It is possible that some of the injected maleylated proteins are taken up by dendritic cells and...
that they contribute towards the priming of T cells. However, data presented here indicate that maleylated proteins can also bind to B cells, macrophages and to certain dendritic cells. This raises an important question about the basis of T cell responses observed in this study.

Priming of T cells by dendritic cells is well known [Inaba et al., 1990; Levin et al., 1993], but the scenario remains controversial regarding B cells and macrophages. It has been shown that lymph node cells from antigen primed IgH deficient mice show lower proliferative responses to immunizing antigens, than those from the wild type. Based on this it has been argued that B cells play a role in T cell priming, but more recently this finding has been shown to be antigen dependent (i.e., for some antigens there was a difference while for others there was not) [Constant et al., 1995]. However, the amplification of T cell responses may be a B cell dependent phenomenon, and this possibility has not been explored. Contradictory reports also exists regarding the role of macrophages in T cell priming [Miyazaki et al., 1993; Unanue et al., 1987].

A relative predominance of type-1 T cell responses in mice immunized with maleylated proteins over those immunized with their native counterparts was observed. Antigen presentation through B cells has been argued to lead to a type-2 T cell response as compared to the presentation by dendritic cells and macrophages [Stockinger et al., 1996]. Dendritic cells and macrophages have been shown to prime T cells to a type-1 T cell phenotype [Hsieh et al., 1993; Macatonia et al., 1995]. In other studies it has been shown that antigen presentation by dendritic cells favors the synthesis of IgG1 and IgG2a, a relatively type-1 T cell response as compared to antigen presentation by macrophages which favored the synthesis of IgG1 and IgE, a type-2 like T cell response [Becker et al., 1994]. It is thus possible that dendritic cells prime for type-1 T cells better than macrophages and B cells, while B cells prime for a better type-2 T cell response than dendritic cells and macrophages. Based
on these facts, it is possible that the immunomodulatory effect of maleylated antigens observed in the present study may be predominantly a consequence of dendritic cell mediated antigen presentation rather than macrophage mediated antigen presentation. However, the role of the B cells can not be ruled out during the whole process.