made in the model (*3*, *4*). These values could change as more definitive physical measurements are made at Jupiter. The electron energy is limited by the maximum motional potential of Io. The energy scales with the equatorial surface magnetic field B at Jupiter: $E_{max} = (B/7 \text{ gauss}) \times 670$ kev. The fraction of this potential available to accelerate electrons depends on the thermal electron density surrounding Io. A density lower than about 10 cm^{-3} would decrease that potential below 300 kev and a density of 100 cm^{-3} or more would give the maximum potential (for an electron temperature of 10^5 °K). In the model discussed here the emitted flux is limited by the photoelectron emission current density at the surface of Io (3×10^{-7} amp m^{-2}) for a yield function of 0.01 electron per photon. If this yield function is higher, then the flux could be increased. Also, if Io has a significant atmosphere the flux would be controlled by the photoionization rate, which would yield a higher flux. An atmosphere of Io could also provide the conductivity required at Io by this model.

Other consequences of the electrons related to Io which may be observable by the Pioneer spacecraft include optical emissions and atmospheric heating. The ultraviolet photometer has channels to observe Lyman alpha emission and the He line at 584 Å (*14*). Shawhan (*5*) estimates an upper limit to the intensity of 10^3 to 10^4 kilorayleighs at the foot of the Io flux tube covering 10^4 km^2 (10^{-6} of the surface of Jupiter), and this might be observable in the dark atmosphere. Also, the energy from the beam of precipitated electrons that is not lost through other emissions could heat the atmosphere down to the cloud tops. An upper limit to the energy input is 8×10^4 erg cm^{-2} sec^{-1} locally, which exceeds the solar input by an order of magnitude (*5*). Perhaps a resulting hot spot would be observable with the infrared radiometer (*14*) since it has a resolution of about 0.01 R_J.

The Pioneer 10 and Pioneer 11 flybys of Jupiter offer a unique opportunity to test the many theories and models concerning the giant planet and its peculiarities. We make our predictions in this spirit and look forward to the experimental results, which will establish values for many parameters up to now estimated with great uncertainty.

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β_2-Microglobulin: Association with Lymphocyte Receptors

Abstract. *β_2-Microglobulin (β_2m) is a low-molecular-weight protein constituent of lymphocyte membranes. Amino acid sequence analysis has revealed a high degree of homology between the β_2m and certain regions of immunoglobulin molecules, suggesting a possible recognition function for the β_2m, in analogy with the immunoglobulins. The data presented demonstrate that highly specific antiserum against β_2m blocks lymphocyte reactivity against allogeneic cells in mixed leukocyte cultures and against phytohemagglutinin, both of which processes presumably function via a cell surface receptor on thymus-derived (T) lymphocytes. There is very little inhibition of T lymphocyte rosette formation with sheep red blood cells. The findings suggest a possible relation between the β_2m and recognition units on the T lymphocyte surface.*

β_2-Microglobulin (β_2m), a protein whose molecular weight is 11,600 (*1*), is associated with the outer membrane of many cells including lymphocytes (*2*), the main effector cells of the immunological system. The amino acid sequence of β_2m shows striking homology with certain parts of the immunoglobulin polypeptide chains (*3*). Given the importance of the immunoglobulin molecules in recognition processes of the immune system, an evaluation of the possible role of β_2m in this perspective seemed indicated.

We have attempted to evaluate possible receptor functions for β_2m on lymphocytes by studying the ability of highly specific antiserums to β_2m (anti-β_2m) to block recognition by lymphocytes; all studies were performed with rabbit antiserum to human β_2m and human lymphocytes. We have used two assay systems, each involving presumed thymus-derived (T) lymphocytes: (i) lymphocyte proliferation in vitro as measured by either the mixed leukocyte culture (MLC) test (*4*), which is an in vitro model of the recognition phase of the immunological reaction leading to homograft rejection (*5*), or by phytohemagglutinin stimulation; and (ii) rosette formation of lymphocytes with sheep red blood cells (SRBC's) (*6*). In MLC tests, as upon stimulation with antigens to which the donor of the cells is sensitized, specific clones of cells respond, different clones responding to different antigens (*7*). Phytohemagglutinin is considered a nonspecific stimulant; that is, there is no such clonal response. Similarly, SRBC rosettes in man are thought to be formed by all T cells.

Antiserums directed at cell surface components have been used in a variety of studies aimed at understanding lymphocyte cell surface topography (*8*) or the biological role of membrane components (*9*). Antibodies bind to the exposed membrane antigen against which they are directed and in addition can sterically block other very closely juxtaposed membrane components. Such binding could prevent both these structures from reacting with other antiserums, and possibly interfere with their biological function.

Our results show that antiserum to β_2m inhibits MLC reactivity, as well as the reactivity of lymphocytes to other mitogens, but does not interfere to the same extent with SRBC rosette formation.

Antiserum to human β_2m was pro-

duced in New Zealand rabbits by four biweekly injections of 1 mg of purified β_2m for each injection. The β_2m was purified by the same procedure used for α_2-microglobulin (*10*). The antiserum was absorbed with normal human serum, normal human urine, pure human lysozyme, and red cell membrane ghosts (*11*). The absorbed serum gave a single precipitin band with purified β_2m and with whole human serum from kidney transplant recipients which contains high levels of β_2m. Antiserums from several rabbits were pooled; this pool was used in all experiments after the complement was inactivated by heating at 56°C for 30 minutes.

In MLC tests, peripheral blood lymphocytes of one individual (for example, A), are incubated with mitomycin C–treated (m) "stimulating cells" of an allogeneic individual (for example, B_m) in the MLC AB_m. If the responding lymphocytes of individual A recognize foreign major histocompatibility antigens on the cells of B, then A cells will enlarge, incorporate thymidine, and divide. The reaction is assayed by studying the incorporation of [^{3}H]thymidine (^{3}HT) into the culture; if more ^{3}HT is incorporated (counts per minute) into the AB_m culture than into an isogeneic control culture (AA_m), then recognition has taken place. The details of the culture system have been described (*12*). Lymphocytes can also be stimulated to thymidine incorporation and division by antigens such as purified protein derivative of tuberculin (PPD) if the donor of the cells is sensitized to that antigen, or by mitogens such as phytohemagglutinin. Antiserum to β_2m was added to the responding cells at the dilutions given and incubated for 2 hours at 37°C before the addition of allogeneic cells, soluble antigen, or mitogen as a stimulus. The cells are incubated with the antiserum throughout the culture period.

A representative experiment testing the effect of anti-β_2m on MLC reactivity and stimulation by phytohemagglutinin is given in Table 1. In both the MLC and phytohemagglutinin reactions there is extensive reaction as assayed by ^{3}HT incorporation in the absence of antiserum. Addition of anti-β_2m at a dilution of 1 : 100 abrogates MLC reactivity and results in a highly significant reduction of phytohemagglutinin reactivity. Higher dilutions of the antiserum have a progressively weaker effect. In 20 different MLC mixtures, the 1 : 100 dilution in all cases depressed reactivity by more than 95 percent; the effect of the 1 : 1000 dilution varied in that MLC reactivity was in 17 of 20 cases still significantly suppressed (maximum, 88 percent), whereas in other cases there was no effect. Reactivity of 16 different responding cells tested with phytohemagglutinin was decreased between 60 and 81 percent by the 1 : 100 dilution of the antiserum; the 1 : 1000 dilution was still significantly suppressive in 13 of 16 cases (maximum, 56 percent). Normal rabbit serum tested at a 1 : 50 dilution did not significantly depress reactivity in MLC or to phytohemagglutinin as compared with normal control human serum. This control finding as well as the ability of purified β_2m to absorb out all cytotoxic activity from the rabbit antiserum to β_2m suggests that the specific antibodies to β_2m are responsible for the blocking.

Table 1. Inhibition of lymphocyte reactivity in mixed leukocyte cultures and to phytohemagglutinin (PHA) by antiserum to β_2m.

Responding cell donor	Stimulus	Incorporated [^{3}H]thymidine (count/min ± S.D.) at dilutions of anti-β_2 in culture		
		None added	1 : 100	1 : 1000
A	None	159 ± 72	65 ± 18	316 ± 87
A	B_m	43,143 ± 2301	159 ± 29	32,149 ± 3469
A	PHA	45,915 ± 1893	16,891 ± 4602	43,283 ± 7449
C	None	217 ± 24	50 ± 3	723 ± 763
C	B_m	19,061 ± 1657	97 ± 26	5199 ± 2003
C	PHA	56,326 ± 625	13,756 ± 778	29,917 ± 3297

In initial experiments we have found that the anti-β_2m is as effective at inhibiting the proliferative response of sensitized lymphocytes to PPD as it is in its MLC inhibition.

Human lymphocytes which have receptors to form SRBC rosettes are operationally defined as T lymphocytes. Lymphocytes purified from human peripheral blood on Ficoll-Isopaque gradients are incubated with SRBC's (*6*), and the percentage of rosettes (lymphocytes with more than three SRBC's attached) are counted. To test inhibition, antiserums were incubated with the lymphocytes before addition of the SRBC's and then left in the incubation mixture. Alternatively, the cells were washed prior to addition of SRBC. The results were similar under both protocols. In seven experiments an average of 20 percent inhibition of rosette formation occurred with the use of a 1 : 100 dilution of the anti-β_2m. As a strong positive control we used an antiserum prepared in goats against human thymocytes. This antiserum at a dilution of 1 : 100, which is not cytotoxic to the lymphocytes under the complement-free conditions of the assay, inhibited more than 97 percent of the rosette-forming cells; the potency of this antiserum to block MLC was equal to or somewhat less than the anti-β_2m.

Cells were tested at various times of culture for their ability to exclude eosin, an assay of cell viability. The presence of the specific anti-β_2m did not result in significantly increased cell death (cell number or percent viability), as compared with the presence of normal control serum. It is possible, despite these studies demonstrating the relative lack of cytotoxic potency of the anti-β_2m against the cultured lymphocytes, that a small subpopulation of the lymphocytes is lysed which cannot be identified within the error of the cytotoxicity assay. This could explain the inhibition of MLC, PPD, or phytohemagglutinin stimulated cultures. This explanation cannot be ruled out; however, if true it would represent a most interesting selective effect of the anti-β_2m.

The highly significant inhibition by the anti-β_2m of lymphocyte response in MLC and to PHA, functions presumably mediated through receptors on the lymphocyte surface, suggest a relation between the β_2m and the receptors. We favor the interpretation that the antiserum blocks the β_2m exposed on the surface of the responding lymphocyte without killing the cell. The β_2m could then be the recognition unit itself or a component of a larger recognition complex in the membrane. The observation that MLC, phytohemagglutinin, and PPD reactivity are all blocked by the anti-β_2m would be consistent either with a single receptor complex which functions for all these stimuli or with different receptor complexes. Again, we cannot rule out that the β_2m is located in close proximity to the receptor site. Alternatively, one could argue that the β_2m is a component of the stimulatory antigens in the MLC test; this would not, however, explain the blocking of PPD and phytohemagglutinin reactivity. Mechanisms could be suggested whereby the anti-β_2m blocks reactivity to phytohemagglutinin.

for example, without blocking the phytohemagglutinin receptor. Although the quantitative aspects of anti-β_2m blocking of SRBC rosette formation needs further investigation, it may be that the receptor for SRBC is distinct and distant from that of the β_2m. Poulik *et al.* (*13*) and Peterson *et al.* (*14*) have recently obtained data suggesting a physical association between the serologically defined antigens of HL-A, the major histocompatability complex in man, and β_2m. In that context, our findings support the concept that phenotypic products of the HL-A chromosomal region may function in recognitive processes.

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Aggregation of HL-A Antigens at the Lymphocyte Surface Induced by Antiserum to β_2-Microglobulin

Abstract. *Rabbit antiserum to human β_2-microglobulin followed by goat antiserum to rabbit immunoglobulin induces aggregation of β_2-microglobulin at the lymphocyte surface, as shown by immunofluorescence and by acquisition of resistance to lysis by complement. This treatment also affects all HL-A antigens of the cell, which cap together with β_2-microglobulin, so that the cell becomes resistant to lysis upon addition of antibodies to HL-A in the presence of complement. These findings suggest that a physical linkage exists between these two classes of polypeptides at the surface of the living cell and is in agreement with recent biochemical data obtained by others with cell membrane preparations.*

It is generally accepted that many protein components of the cell membrane are floating in the lipid bilayer, where they are oriented according to their physical characteristics (*1*). Antibodies directed against some of these products are potent and specific agents for rearranging cell surface components without impairing cell viability. In fact, antibody-mediated aggregation of molecules at the outer layer into "caps" and "spots" has become an important technique for exploring the topography of the cell surface (*2*). With this method we have proved definitively that each one of the three pairs of genes of the HL-A chromosomal region of man, which are expressed as "serologically defined" antigens (SD), are responsible for the synthesis of distinctive physical units that do not interact with each other. Thus the six HL-A antigens (of heterozygotes at the three SD loci) move independently at the cell surface and do not cap together (co-cap) (*3, 4*). Surface immunoglobulins of B (marrow-derived) lymphocytes also do not co-cap with transplantation antigens (*2, 5*). Usually, labeled reagents are employed, and the rearrangement of molecules is visually studied by immunofluorescence microscopy. Bernoco *et al.* (*3*), however, have also observed that formation of complexes of HL-A antigen plus antibody to HL-A plus antiserum to human Ig (immunoglobulin) at the lymphocyte surface make the cells specifically resistant to complement-dependent lysis, even upon addition of new antibody and excess complement, for all HL-A determinants carried by the molecular population which has been aggregated.

By contrast, the cell is normally susceptible to complement-dependent lysis for the residual HL-A antigens. For other systems similar phenomena have been described as "antigenic modulation" (*6, 7*). Actually, fixation in vitro on the surface of living lymphocytes of antibodies to HL-A antigen (and by implication of antibody against some other membrane components) is labile and initiates a complex chain of events (*8*). Of particular significance for this presentation are the following. (i) "Desensitization" of the cells, during incubation at 37°C, becomes progressively resistant to lysis upon addition of complement alone (*9*). This is due in part to microaggregation of the antigen, in part to loss from the cell surface of the antigen-antibody complex by endopynocytosis, shedding, and, later, peptic digestion (*10*). Usually the lost antigen is replaced at the same rate by virgin molecules, and the cell is still lysed by addition of further antibody and complement. (ii) The rapid formation of caps and spots by the two-step procedure (addition of human antiserum to HL-A and then antiserum to human Ig) leaves large areas of the cell surface totally deprived of that antigen, as seen by immunofluorescence, and paralleled by resistance to complement-dependent lysis. (iii) HL-A molecules lost by two-step addition described above are replaced by new ones (recovery) if the cells are incubated in nutrient medium at 37°C for 8 to 12 hours. (iv) Recovery requires active protein synthesis and is inhibited by cycloheximide and other antimetabolites.

With this background information concerning HL-A behavior, we have studied, in the living cell, the behavior of β_2-microglobulin (β_2m) which is present on the surface of many types of cells, including lymphocytes (*11, 12*). Particularly, Poulik (*13*) has shown that rabbit antiserum to human β_2m which has been appropriately absorbed, allows the detection of β_2m at the lymphocyte surface by immunofluorescence (indirect method) and induces cell lysis upon addition of rabbit complement.

For the reagents and techniques used in the experiments presented below see (*14*). Desensitization of lymphocytes coated with antiserum to β_2m (anti-