HAEMATOLOGICAL CHANGES IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION. PART I: REVIEW ARTICLE

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SUMMARY

This review attempts to put together the changes in the blood and bone marrow observed in those who are infected with human immunodeficiency virus (HIV). These are contribution of many published and unpublished data and experience on; blood counts, blood film and bone marrow films prepared and stained by May-Grunwald-Giemsa or Leishman stain. Some changes in haemostasis are also included. The salient changes are cytopaenias; leucopaenia, anaemia, thrombocytopenia, and bone marrow hypoplasia, although the latter occurs, it is found in a minority of cases. Other changes include myelodysplasia, functionally defective cells, and enhanced bleeding tendency particularly in those with bleeding defects. There are also malignancies associated with HIV infection such as Kaposi's Sarcoma and malignant lymphomas. The pathogenesis of these events are multi-factorial, varied and involve; killing of cells by the virus, syncytial formation by the cells, destruction of the stem cells, immune and drugs effects. These mechanisms are modified by factors of viral, host environment and their interactions. Changes are commonly found in patients with acquired immunodeficiency syndrome (AIDS) but can be seen in some cases anytime during the course of the disease. Once developed the changes are progressive. The management of these complications remain individualised and symptomatic. Treatment trials with the haematopoiesis growth factors, particularly colony stimulating factors are producing some encouraging results. However other cytokines, for example, interleukin-6 may be having untoward effect such as association with the causation of Kaposi's sarcoma and the malignant non-Hodgkin's lymphomas. While standard approaches to the management of the malignancies tend to be the practice, adjustments are usually necessary in most patients.

INTRODUCTION

The natural history of the human immunodeficiency virus (HIV) infection is characterised by perturbations of literally every type of cell, tissue, organ and system in the human body(1). The haematological cells are mainly affected as some of its cellular components, the lymphocytes are prime target for the HIV(2). Therefore the period of HIV infection is punctuated by appearances of a variety of clinical and laboratory manifestations of the changes in the haematological systems(3). Other viruses, particularly HIV-2, in the same classification as the HIV-1 have been shown to cause similar effects and immunodeficiency syndromes (AIDS)(4). However, herein HIV refers to HIV-1. Further, the aspects of haematology reviewed are the cellular components including the bone marrow and aspects of haemostasis. These are; red blood cells, the haemoglobin and white blood cells and their components of neutrophils, eosinophils, basophils, monocytes, and lymphocytes, and platelets. Films of bone marrow aspirate, trephine bone biopsy and blood including the differential white cell counts. Some citations on the functional or qualitative aspects of these cellular components are mentioned. Also included are some commonly encountered changes in haemostasis. Mechanisms leading to the changes are alluded to although not in depth as most of the pathophysiological mechanisms remain vague and many are still in the investigation stages. The role of cytokines and growth factors in these changes, HIV associated malignancies and their management are briefly discussed.

The erythrocyte sedimentation rate and rouleaux formation as part of haematological evaluation are alluded to. The factors which modify the severity and time of onset of these disturbances are briefly discussed. About 90% of the people infected with the HIV develop a haematological abnormality during the course of the disease(4-5). Some people develop haematological features earlier than others(6). It appears, therefore, that there are factors; viral, host, and their interactions that determine the time of onset, the severity, and the type of haematological defect manifested(7). These aspects are discussed as well.

Sociodemographic factors: HIV infected patients in the developing countries tend to develop haematological changes earlier in infection than their counterparts in the developed part of the world, the route of contracting the HIV infection notwithstanding(8). This is postulated to be due to the prevalence of infectious diseases like malaria, hookworm, and tuberculosis, which have their own deleterious effect on the haematological system(8,9). In addition these diseases are aggravated by the HIV infection(10,12). Overall HIV infection has been shown to
exhibit its effects disproportionately in racial and ethnic minorities in the United States of America, and poor communities in other parts of the world(10,11). In the USA despite the clinical recommendations that uniform standards be followed regardless of the sociodemographic factors such as sex, race, and age, there is still disparity in, for example, anti-HIV drug Zidovudine (AZT) usage(12). AZT has significant influence on the haematological changes observed in HIV infection. Age at the time of infection by the HIV is the single most determinant of the time of evolution to AIDS, in for example haemophilics(9,13). Pre-adolescence and young adults progress less rapidly than do those infected after the ages of 45/9,13. The plausible explanation to this is that the younger individuals have a bone marrow that has a larger reserve and can cope better if insulted.

These additional diseases would be expected to be transmitted to the HIV infection altering the overall expression of the disease. There are fewer females with haematological changes before the AIDS phase than there are males(14, author's unpublished data). This may be due to the fact that given the same incidence, the females get to the AIDS phase faster than males or the females may actually be less prone to developing some haematological complications(15). However our series show more females with immune thrombocytopenic purpura(1TP) than males despite the fact that we have more males with haematological changes (personal unpublished data). The differences observed in the racial distribution of the haematological changes are probably due to economic and social factors and to poverty rather than race itself(16,17).

Pathogenesis of the haematological changes: The mechanisms leading to these changes are complex, variable, and as already observed above, depend on a multitude of factors related to the HIV virus, the infected host and their interaction. Generally, the mechanisms include: direct killing of the cells by the HIV, synovial formation of the cells, infections, destruction of the stem cells, and immune effects, including autoimmune destruction of the normal immune regulation pathways(16). Underlying factors in these mechanisms are the viral load and the progressive nature of the HIV infection(18).

Other modifying factors as cited above include sociodemography of the affected person and interventional measures such as drugs that can be toxic to the progenitor cells and directly myelosuppressive(18). The HIV associated malignancies; such as malignant lymphomas are causes of bone marrow dysfunction(19). Infections, particularly tuberculosis which is common in some HIV hyperendemic areas, cause haematological abnormalities (10,11). There is considerable work on the role of growth factors, cytokines and other molecular factors such as Granulocyte Colony Stimulating Factor (GCSF), Granulocyte Macrophage Colony Stimulating Factor (GMCSF), Macrophage Colony Stimulating Factor (MCSF), Tumour Necrosis Factor Apha (TNFa), Interleukin 3 (IL-3), and IL-6, in HIV infection related haematological changes and their management (20,21). It is envisioned that these growth factors would have a role due to their modes of action of stimulation, proliferation, differentiation, and activation on the bone marrow progenitor and relatively mature cells(21,22). For example, it has been shown that there is decline in the GMCSF production correlating with the HIV load and the production of CD4 T-lymphocytes(23). This results in cytopaenia and bone marrow suppression(23). IL-3 was initially described as a T-cell lymphocyte stimulator, therefore its deficiency can be correlated with the reduction in T Cells numbers which is a consequence of HIV infection(24).

SPECIFIC HAEMATOLOGICAL CHANGES

Erythrocyte sedimentation rate (ESR): The ESR is commonly raised in HIV infection, but is always raised in AIDS(25). Raised levels reflect the presence of active disease associated with release of reactive proteins and or gammaglobulins(26). In HIV infection the raised ESR levels are explained on the basis of increased production of polyclonal antibodies and other associated infective processes and lymphoproliferative disorders(32,40,43).

Rouleaux formation: This is one of the most striking features of the blood and bone marrow film examination findings(25). It reflects the presence of proteins surrounding the red blood cells(26). HIV infection affects the B-lymphocytes as well(19). This leads to abnormally increased production of gammaglobulins(26). Other associated bacterial infections give rise to acute phase proteins further causing raised ESR and rouleaux formation seen in the blood and marrow films(25). Other film changes include red blood cells anisopiktocytosis, white cells, and platelets morphological features of myelodysplasia (25).

Red blood cells (RBC): There are both qualitative and quantitative alterations in the RBC. These are a consequence of dyserythropoiesis due to the HIV itself; other infections, drugs and the interaction of these factors. The resulting dyserythropoiesis has been likened to the type which occurs in orthotopic liver transplant(27). The morphological film appearance includes; anisopiktocytosis, macrocytes, particularly in patients receiving AZT, RBC fragments, and oval shaped cells(28). The life span of such dysplastic cells is usually shorter as they are removed from the circulation by the reticuloendothelial cells and by intramedullary destruction. Abnormal utilisation of iron, folate, and vitamin B12 noted in HIV infection contribute to the abnormal erythrocytes and their precursors. The clinical and laboratory consequences of all these are the anaemia(25,28). Anaemia is the commonest haematological feature encountered in HIV infection and 90% on AIDS cases have anaemia of some degree (28). The causes of anaemia are multiple and include viral invasion of the marrow cells with subsequent destruction or dysfunction of the haemopoietic cells. Drug induced anaemias are common. AZT and other dideoxy nucleosides are directly toxic to the marrow cells and 28-30% of
patients taking AZT develop anaemia(28). Other drugs such as sulphonamides and some antituberculous drugs are implicated in myelosuppressive effects. Malignancies such as lymphomas and Kaposi's sarcoma affect the normal activities of the marrow directly or through the cytototoxic drugs used in their treatment(29). Anaemia due to chronic disease or stress, with which the HIV infection is associated are contributory. Low levels and poor utilisation of folate and cobalamin have been reported in HIV infection. Dowigs et al (30) observed significant differences in Vitamin B12 and folate levels in different stages of HIV infection. Hansen et al(31) in their study demonstrated low levels of holotranscobalamin and holohaptocorrin in AIDS patients. Immunological mechanisms have been demonstrated in the causation of anaemia. These include indirect mechanisms at the stage of AIDS by autoantibodies directed against antigens in the red cells(32). Red cell antibodies and positive direct Coombs' test have been demonstrated. Anti-i and anti- U associated with haemolysis in HIV infection have also been demonstrated. In addition circulating immune complexes suppressing the bone marrow progenitor cells and the marrow stroma of HIV infected cases have been illustrated(32). Other mechanisms in the causation of anaemia include, the HIV directly impairing the survival and proliferative capacity of the haematopoietic progenitor cells(33). Infection by the HIV of the marrow and other cells which produce growth factors and cytokines with subsequent reduction of the factors effect on progenitor cells, partly explains the resulting anaemia(34). Other infections such as malaria, tuberculosis, amebiasis, as noted above, contribute and complicate the anaemia. The management of anaemia should depend on treating the primary cause. However, so far, the major approach to the pathology of the anaemic patients has remained supportive.

The available results on the use of erythropoietin and other colony stimulating factors are encouraging but still in the early stages(34).

**White blood cells (WBC):** The leucocytes' central role in the cellular and humoral defence against infectious agents puts them in the direct path of the HIV infection(12). But the changes observed appear to be more than can be explained by WBC functions against infectious incursions(35). These changes affect functional and numerical aspects of the WBC. The mechanisms giving rise to these changes are many and some are ill understood(36).

**Lymphocytes:** The HIV is primarily lymphotrophic and specifically to the CD4+ lymphocyte subtype(1,2). In the early stages of infection the predominant cells affected are the lymphocytes(34). The progression of the HIV infection can be assessed by monitoring the lymphocyte counts(36,37). The HIV infestation of the lymphocytes, results in the death of the infected cells(37). In addition, the functions of lymphocytes are rendered defective by the viral activities, for example, the rise in levels of polyclonal antibodies observed in most patients is a result of abnormal production by the B-lymphocytes.

**Neutrophils:** Neutropenia is frequently noticed in HIV infection(37). The pathogenesis of neutropenia is multifactorial, including immunological factors. Neutrophil bound immunoglobulins (NB1g) in HIV infected individuals, both neutropenic and non-cytopenic have been clearly demonstrated(36,38). Specific autoantibodies against neutrophils have been noted in early infection and their prevalence correlates with the disease progression(37). However, not all the cases where these antibodies were demonstrated showed neutropenia. This suggests other possible mechanisms for the neutropenia(37). The direct invasion of the neutrophils and their marrow precursors cause cytolysis and abnormal cells which are functionally defective(39). AZT associated neutropenia is due to it's myelosuppressive effect.

The deficiency of G-CSF, M-CSF, IL-1, IL-2, IL-3, IL-4 and IL-6 in the bone marrow of the infected patients is associated with neutropenia(38). Neutrophil dysfunction is attributed to lack of phagocytosis and production of toxic substances as a result of invasion of the cells by the virus(38, 40). HIV direct cytotoxicity and alteration of the mechanisms that lead to destruction of the organism by the neutrophils have been demonstrated by Gabrilovich et al(41). Some functions revert to normal after in vitro exposure to GM-CSF(39). This shows that there is interference by the HIV infective process in the production or function of the endogenous colony stimulating factors (CSF)(39, 42).

**Eosinophils and Basophils:** Numerical reduction and functional deficiency are some of the changes that have been demonstrated in these cells(37,39). However, these changes tend to be obscured in many HIV infected cases with allergic skin diseases.

In our experience, relative eosinophilia has been more commonly observed than eosinopenia(25). Basophil changes are better observed in the bone marrow where they are more clearly demonstrated. Again basophilia tends to be the more frequent finding in those with skin lesions in HIV infection(25).

**Monocytes:** Monocytopenia is the commonest change noticed in the monocytes in HIV infection. It is observed in 12-14% of cases and in those patients who have bone marrow examined for monocytes the incidence is high 35-43%(43). These changes are due to the suppressive effect of the virus on the synthesis of monocytes(43). The cells of the monocyte lineage are important targets for the replication of the HIV(44).

Together with the macrophages their infection represents the majority of cells infected by the virus in some body compartments like the central nervous system(44). Further suppression of HIV replication in the granulocytes monocytes type macrophages by GM-CSF explains the effect the virus has on these factors. There is a decline in the GM-CSF production and this correlates with the viral load(45). Exogenous GM-CSF given to patients elicit an increase of white cells particularly the neutrophils and the monocytes in the AZT induced cytopenias(46).
Platelet: The conspicuous change observed in the platelets is thrombocytopenia. Functional defects however, are also frequently seen. Thrombocytopenia is probably the most well recognised occurrence in HIV infection (47-48). In some cases the features associated with thrombocytopenia are the preceding clinical symptoms and laboratory signs in the HIV infected cases. The aetiological causes of thrombocytopenia include the causes of pancytopenia in HIV infection (48). Sacchi et al in a study of bone marrow in HIV infection with peripheral thrombocytopenia demonstrated small dysplastic changes in the megakaryocytes that are incapable of producing sufficient number of platelets (48). These type of megakaryocytes undergo intramedullary destruction. Studies on the most recently infected patients show accelerated platelet destruction and sequestration in the spleen, while the more advanced and frank AIDS cases demonstrated platelet production defects (47,48). The mechanisms of this destruction is not very clear, however, non-specific deposition of circulating immune complexes on platelets, presence of specific antiplatelet antibodies and direct infection of the megakaryocyte by the HIV resulting in decreased production (49). Antibodies to the HIV was eluted from the platelets of patients with thrombocytopenia (48). These two features suggest direct viral invasion of the platelets and the megakaryocytes leading to their destruction. The classical immune thrombocytopenia (ITP) in HIV infection has been demonstrated by several workers, and it appears that ITP is part of the clinical spectrum of AIDS related disorders (48). Levels of platelets associated IgG and complement higher than the levels found in classical immune thrombocytopenic purpura (ITP) have been demonstrated in homosexual patients with thrombocytopenia. Further, the levels were higher than the levels in homosexual controls (48). Costello et al reported the presence of autoantibodies in platelet eluate, these were antibodies directed against platelet glycoprotein (GP) Ib/IIa complex in AIDS patients with ITP (50). Eluates from platelets of HIV related ITP contained IgG reacting to HIV GP160/120 (49,51). This suggests some molecular mimicry between HIV GP160/120 and platelets (52). Platelet membrane antibodies in homosexual patients with ITP was reported to react against a platelet membrane antigen, suggesting the presence of a platelet antibody (50). The presence of antibodies to platelets suggest a defect in autoimmunity or non-specific HIV induced B cell stimulation (45). The treatment of thrombocytopenia, like other abnormalities associated with HIV infection should aim at curtailing the replication of the HIV. This would reduce its direct activities in suppressing the haemopoiesis and altering of the immune mechanism. Specific antiviral therapy AZT is the most successful so far in correcting thrombocytopenia in some cases (50). Splenectomy is reported to be safe and effective in HIV related immune thrombocytopenia. This is so to thrombocytopenias which present like typical ITP in non HIV infected individuals (51,53). Conventional prednisone therapy is also effective in most cases, however when the drug is stopped relapse is noted. The risk of infection when using steroids in these patients is, however a pertinent concern and has to be measured against the benefits. High dose intravenous immunoglobulins may produce a significant increase in platelet count. Platelet transfusion may be used as a stop gap to enable invasive procedures associated with bleeding he undertaken (55).

REFERENCES

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